

More than facts, judgments

THE idea of a Science Court has gained a modest amount of momentum in the United States (*Science*, August 20, p 653; *Nature*, October 7, p 454). It would concern itself solely with providing "factual statements of the highest presumptive validity consistent with time constraints". For this, an adversary procedure would be used and a panel of judges with skills in adjacent areas would be expected to examine statements and, if necessary, write their own opinions on contested matters. Issues which might come early to such a court are: should fluorocarbons be banned because of their effect on the ozone layer? Should water supplies be fluoridated? Should a specific nuclear plant be licensed?

The working party's interim report (in *Science*) has the clear implication in its opening paragraph that a Science Court, by sticking closely to what can be factually established, will be able to hand over something useful to the decision-makers of society, who will then incorporate social value questions. A scientific fact is defined as a result, or an anticipated result, of an experiment or an observation of nature. On the other hand, the report continues, "we have no illusions that this procedure will arrive at the truth, which is elusive and tends to change from year to year." Thereby the science court neatly ducks the issues and relegates science to a backroom, purely technical rôle.

The scientist is most unlikely to be able to deliver to the decision-maker any useful sort of factual statement, because he is hardly going to be allowed to perform the appropriately large experiment or observation. All he can generally supply in the way of facts is some results from pilot projects, some calculations which may be relevant and so on. What the good scientist should also be competent to provide, however, is inference, and this, albeit tentative and hedged-about, is what the decision-maker needs and what the science court seems to avoid.

Factual statements of the highest presumptive validity would merely be about rats, about rocket samples, about tensile strengths. Those involved in public policy need to know whether, in the scientist's best judgment, such statements can be generalised. Intelligent customers for these sorts of judgments know full well that scientific 'truth', being a whole level higher than facts, is often every bit as elusive and changeable as political and economic 'truth'. But they still expect the scientist to go beyond the solid ground of his facts.

It is difficult to see that a science court has anything new to offer if it steers clear of this territory—indeed the exercise could be positively dangerous in that others, less qualified, may be tempted to draw their own conclusions from such handily accumulated and uninterpreted facts. □

More than judgments, action?

THE best debates never die. As the arguments about nuclear power continued in Britain last week with the resumption of the SGHWR enquiry by the House of Commons Select Committee on Science and Technology, life was again being breathed into a still more fundamental (though still less newsworthy) debate—the centuries-old argument about the depletion of the Earth's natural resources. It came with the publication of *Materials and Energy Resources*, the report from a three-year-old working party on resources embraced by the UK Institution of Chemical Engineers (I.Chem.E.).

At £5 it is a costly two-score pages, not helped by the I.Chem.E. wanting, apparently, to have its cake and eat it. It is both seeking to stimulate reasoned discussion on crucial resource problems and trying to get something done about them. Thus, while the report rightly points out the vital need to see materials and energy problems as two sides of the same coin, and traces the broadly-based working party's own valuable assessments of these problems, it is also being presented as an attempt to influence policy. Not, though, general resources policy internationally, but specifically, and as a matter of strategic importance, UK government policy.

That is laudable enough, perhaps, but represents a curious

disposition for such a study from such a body. After all, the disciplines it represents and the problems it is tackling are nothing if not international, as are the phenomena like international cartels which it acknowledges. Since it also focuses its attention on a period 10 to 35 years hence (by which time, if the past is anything to go by, the world order ought to have changed), the working party might have done better to look more closely for international solutions.

Not that it has done wrong in sending the report to the Secretaries of State for Energy, Industry and the Environment. But, in spite of what was described enthusiastically last week as a positive response from Industry, the fears being expressed privately that the report could easily lose itself in the bureaucracy are more than justified. The I.Chem.E., which probably wants simply to be a professional group turning out professional work, ought to leave both this problem and the job of actually campaigning for changes in policy to the pressure groups.

The present importance of the nuclear issue, in the UK and elsewhere, is not a little due to fringe groups making effective use of the information and judgment from reputable bodies ill-placed if not disinclined to deploy it themselves. The resources issue ought to be next. The best debates never die. □



Seveso: the aftermath

Alastair Hay reports on developments since a chemical explosion rocked this northern Italian town

THREE months have elapsed since the now notorious Séveso accident of July 10. It occurred when a reactor producing trichlorophenol at the ICMESA chemical plant—owned by Givaudan, a Hoffman-La Roche subsidiary—became overheated: the rapid pressure increase that followed led to the discharge of its contents through a safety valve directly into the atmosphere. Scientists have since been engaged in decontamination work in the area, which was affected by the fallout of TCDD (2,3,7,8-tetrachlorodibenzo-para-dioxin) as the disastrous consequence of that discharge. A considerable amount of disturbing information has now come to light concerning both the operation of the ICMESA plant, and the decontamination procedures adopted.

Givaudan began to produce trichlorophenol at the ICMESA plant only in 1975. The monthly output of the plant was 5 tons but, in producing this quantity of trichlorophenol, the reaction also produced 1.2 kg of dioxin. This, according to an Italian trade union document, is the quantity which ICMESA was required to dispose of every month. It represents a high level of dioxin formation, of the order of 200 p.p.m.

According to a report in *The Times* earlier this week, however, Dr Donald Lee, the British expert now investigating the accident, is thought to have told the Italian authorities that up to 130 kg of dioxin could in fact have been produced in the explosion. His recommendations are said to include the suggestions that residents should have medical tests for the rest of their lives, that the contaminated area should be monitored for decades, that this and a buffer area should be sealed off to all but authorised personnel, and that decontamination procedures should involve the creation of forests and dismantling of buildings rather than the shifting of earth and the washing of buildings.

Although a senior Roche executive says, according to the Italian weekly *L'Espresso*, that the company was producing TCDD deliberately for military purposes, Givaudan has denied the allegation. But Professor Fritz Mori, designer of the trichlorophenol reactor at ICMESA, has referred to the cause of the dioxin formation through a sudden rise in temperature within the reactor and gone on record as saying it would be impossible "unless the re-

actor was being used to produce substances other than the trichlorophenol it was designed to produce". The design of the reactor involved in the accident has come in for severe criticism, for incorporating safety features inadequate for the production of chlorinated phenols.

Information possibly vital to the resolution of the problems of plant operation, design and safety, however, is still not available to the Italian authorities. As for the estimate that the residents of Séveso and its surrounding area will not be allowed to return to their homes for at least a year, this is regarded as optimistic by many scientists. Although vegetation from the less contaminated zone is now being incinerated, no specific plan for removing dioxin from the soil has yet been agreed. Originally, topsoil from the total area contaminated was to have been cleared and processed to remove dioxin. However, some recent tests have revealed dioxin at a depth of 25 cm. The original proposition, which relied on TCDD being close to the surface, is now considered to be too difficult an undertaking, and operations will be confined to removing dioxin from the 30–40 hectare zone most severely contaminated.

Antidote claimed

Givaudan claims to have developed an antidote which will destroy the dioxin, consisting of an olive oil–water emulsion and cyclohexanone. In principle, the dioxin is detoxified by rupture of the linked ring system and the removal of the chlorine atoms by the unsaturated bonds of polyunsaturated fatty acids present in the olive oil. Ultraviolet light absorbed by the cyclohexanone initiates the reaction sequence. However, the Italian authorities, as well as American and British scientists, are sceptical about the success of the Givaudan claim. The Italians and Americans maintain that there is no effective antidote for TCDD. And Professor Derek Bryce-Smith of Reading University says sunlight does not contain a high proportion of ultraviolet in the wavelength range where cyclohexanone absorbs it. Although the photochemical reaction may take place in the laboratory, there is, he says, a real danger of moving dioxin so that it is harder to get to.

TCDD is virtually insoluble in water, and for this reason is not translocated rapidly in soil. Placing a dioxin solvent

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Keystone

Four-year-old Séveso victim

on the soil—TCDD is partially soluble in organic solvents—could obviously assist in moving the dioxin to greater depths. Another complication is the fact that cyclohexanone is itself a dangerous chemical: its flash point, only 63 °C, is well below its boiling point of 155.6 °C; in terms of human toxicity, it is considered a weak narcotic, and it also irritates skin and mucous membranes; the lethal dose for mice is 8,000 p.p.m. in air.

The reports causing most worry, though, are those concerning the results of the blood tests carried out on the ten thousand residents of the Séveso area. Information not yet released by the Italian health officials confirms that 5–10% of the people examined are showing reduced white cell blood counts. Although it is only three months since dioxin was released, and a reduction in blood lymphocyte count is a secondary effect of damage to the immune response tissue—which dioxin is known to affect—these results are causing great concern. The situation is expected to worsen, and more residents may show adverse clinical symptoms; persons already affected could record a further reduction in their white cell count. But some scientists who have recently returned to the UK after visiting Italy feel that symptoms of dioxin poisoning are less serious than was originally feared.

Given the known teratogenic properties of dioxin, 150 women from the Séveso area who were in the first trimester of pregnancy at the time of the explosion have applied for an abortion. By the end of September, 25 women had received government authorisation for the operation, in the face of great opposition from the Catholic Church. Authorisation for another 125 women is also likely.

Health officials have examined a total of 730 pregnant women formerly resident in the contaminated area. The difficulties in securing legal abortions have added considerable impetus to the

campaign for abortion law reform; the Italian parliament is to consider several bills advocating changes.

In view of the known toxicity of TCDD and the knowledge that it is a

constant by-product of the trichlorophenol manufacturing process, many scientists are now beginning to question the need for the continuation of trichlorophenol production. One of the two

The possible alternatives

THE available alternatives to 2,4,5-T are 'Amcide', 'Glyphosate' and 'Krenite'; all three are effective brushwood killers. Amcide has been available in the UK since the early 1960s; Glyphosate is a more recent addition, and Krenite is at present only on sale in the US and West Germany.

These alternatives, when compared with 2,4,5-T, have some definite advantages. Amcide, made by Nissan in Japan, has an LD₅₀ value for rats of 3,900 mg kg⁻¹; Glyphosate, made by Monsanto, has an LD₅₀ value of 4,900 mg kg⁻¹. These toxicity ratings are well below that for 2,4,5-T (LD₅₀ value 300 mg kg⁻¹). Krenite, made by Dupont, has an LD₅₀ value of 24,000 mg kg⁻¹, a toxicity rating one eightieth of that for 2,4,5-T.

The reaction to produce Amcide presents none of the potential hazards associated with trichlorophenol manufacture in terms of toxic by-products. Information has not been made available for Glyphosate or Krenite production. However, in the case of Krenite the US Environmental Protection Agency (EPA) considers its manufacture to conform to accepted safety standards, and in view of its comparatively low toxicity has declared it as safe for use even on land adjacent to domestic water supply reservoirs and streams.

The disadvantages associated with the three alternatives are primarily those of cost. Amcide and Glyphosate are more expensive than 2,4,5-T when the concentrations of herbicide necessary to effect the same plant kill ratio are considered; Glyphosate is nearly five times as costly. Some potential buyers of Krenite consider that it, too, may be expensive when it is introduced on the UK market. The only other problem of any consequence relates to Amcide. In contrast to 2,4,5-T which is absorbed through leaves following spraying, Amcide must be applied to cut surfaces on plants. Its method of application is therefore considerably more labour intensive.

Hexachlorophene, the other major product derived from trichlorophenol, is a general poison effective in the control of gram-positive bacteria. In the cosmetics industry hexachlorophene is used as a preservative. For medical purposes hexachlorophene is used in the control of staphylococcal organisms. The bactericide has four

main uses: treatment of acne and impetigo, cleansing of intact skin around burns or wounds, pre-surgical washing, and cleansing of new-born infants, particularly the umbilical cord.

Its use, however, has been much reduced, and the industry is reported as being able to dispense with it altogether. Chlorhexidine—a bactericide now cleared for sale in the US—is used surgically in the UK as a skin cleanser, wound steriliser and for pre-surgical washing. On the question of the cleansing of the newly-born infant, however, there is a difference of opinion as to which of the two bactericides is the most effective.

Maternity clinics and nurseries are particularly open to bacterial cross-infection by the staphylococcal organisms. One of the most common is *S. aureus*. In the 1940s this organism was responsible for frequent epidemics in nurseries, with a consequent increase in infant mortality. When hexachlorophene was first marketed by Givaudan in the late 1940s it proved to be effective both in the routine containment of *S. aureus* and in controlling the organism in the case of an epidemic. As a result of its efficiency, hexachlorophene rapidly replaced the bactericide 'Triple Dye' in use at that time, to become the most widely used antibacterial agent in nurseries.

Two events in 1971 and 1972, however, caused users to reconsider their judgment. The first was a report by the EPA showing hexachlorophene to cause oedema and hindlimb paralysis in experiments on mice. The second was the death in France of 35 infants following the use of talcum powder containing 6% hexachlorophene. The neurological damage which led to the death of the children was caused by a twenty-fold increase of hexachlorophene concentration in the talc, the result of a manufacturing error.

Many maternity units in the UK have since reduced the amount of hexachlorophene used for infant washes. Currently less than half use hexachlorophene at all. Others rely on alcohol, used either alone or with chlorhexidine. Consultants at one cross-infection laboratory now recommend nurseries to avoid the use of hexachlorophene altogether for routine washes. The reasoning behind

this recommendation lies in the evolution of *S. aureus* itself, which has evolved through several forms since the 1940s and is now active as a complex of *S. aureus*.

The present generation of the bacterium is not causing serious epidemics in British hospitals which are fatal to children. Indeed the risk associated with hexachlorophene use is considered to present a greater threat than that represented by the staphylococcus itself. A further consideration is the fact that many British maternity units use a concentration of hexachlorophene to control *S. aureus* which is too low to kill the bacterium.

In the routine control of *S. aureus* chlorhexidine is as effective as hexachlorophene, and has the added advantage of an LD₅₀ value ten times higher. Chlorhexidine is also reported to be just as efficient in controlling local epidemics of the present milder strain of the bacterium. It has not been tested, however, with more lethal strains of the staphylococcus, whereas hexachlorophene has been shown to control dangerous epidemics of *S. aureus* in the past. Some bacteriologists anticipate that chlorhexidine will prove to be equally effective, but are reluctant to recommend it unequivocally as an alternative until this has been demonstrated conclusively.

Although the principal purpose behind trichlorophenol production is the synthesis of 2,4,5-T and hexachlorophene, the chlorinated phenol is still used to some extent as a slime control agent in the paper-making industry. It has never been as popular as pentachlorophenol, used for the same purpose, and what use it has had has been much reduced, due mainly to the criticisms of the unrestricted industrial use of polychlorinated biphenyls (PCBs) such as DDT, advanced by the environmental lobby.

In common with many industries which discharge effluent directly into rivers, the paper manufacturers have been particularly sensitive to the charge that their plant operating procedures are damaging to the environment. Because of the known risks associated with extensive PCB use, paper manufacturers in the UK have considerably reduced their use of all chlorinated phenol derivatives. Agents now preferred for the prevention of fungal growth include methylene bis thiocyanate together with some organobromine and organosulphur products.

products derived from trichlorophenol—the herbicide 2,4,5-trichlorophenoxyacetic, 2,4,5-T (the other is the bactericide hexachlorophene)—has many formulations which contain the dioxin contaminant. In view of this the Norwegian authorities banned the use of the herbicide altogether in 1973. The US Environmental Protection Agency (EPA) regards 2,4,5-T as a product which may be too hazardous to man and the environment to permit continued use.

In the UK, however, the Ministry of Agriculture, Fisheries and Food's 1976 "List of Approved Products for Farmers and Growers" contains five formulations of 2,4,5-T alone, and eight of 2,4,5-T in combination with 2,4-D (2,4-dichlorophenoxyacetic acid). These products are sold as bramble, brushwood and nettle killers. A new formulation of 2,4,5-T, 'Silvapron T', produced by British Petroleum, is currently on trial in Britain. The herbicide is dispersed in an oil-based mixture which

avoids some of the problems of volatilisation encountered with the traditional water-based mixtures. This BP formulation is restricted to forest areas.

Neither product unique

But neither 2,4,5-T nor hexachlorophene is unique. Alternative herbicides and an equally effective bactericide are available (see box). Because of this, many scientists now question the desirability of using a reaction as dangerous as that involved in synthesising trichlorophenol. They point out that, with one exception, every plant producing trichlorophenol has had a serious accident involving release of dioxin, and injury to workers and the general public. The list of accidents begins with Monsanto (US) in 1949; then comes Badische Anilin und Soda Fabrik AG (West Germany) in 1953, Philips Duphar (Holland) in 1963, Dow Chemical Company (US) in the early 1960s, Coalite and Chemical Products Ltd (UK) in 1968, and finally, Givaudan-ICMESA (Italy) in 1976. Bayer of Leverkusen (West Germany) is the sole apparent exception.

But it is not only scientists who are concerned about the operation of the trichlorophenol process. The Italian authorities have invited Givaudan to build a new chemical plant near Séveso, with the provisos that it utilises reaction processes known to be safe and that trichlorophenol is not produced. Two other manufacturers of trichlorophenol are equally concerned; the Coalite and Bayer plants have both temporarily ceased operation. Additional safety features are to be introduced at Coalite as a result of recommendations by the UK Health and Safety Executive, but forty-nine of the fifty workers have refused to work there under any circumstances.

The trichlorophenol manufacturing process is only one of the potentially

hazardous operations which will now come under closer scrutiny as a result of the devastation at Séveso. There are few processes which can have the unique record of an almost one-to-one ratio of accidents to production sites. However, there may be some where the value to society of the products manufactured is not sufficient to justify continued operation. The disaster in Italy will provide the backcloth for the debate which could help to resolve these issues.

● Yet another release of a dangerous chemical occurred in Italy on 26 September. This time between 10 and 30 tonnes (according to different sources) of arsenious oxide "escaped" from the ANIC chemical plant—a subsidiary of the Italian state fuel concern ENI—near Manfredonia on the Adriatic coast.

Over fifty people including two infants have been hospitalised with suspected arsenic poisoning (LD_{50} for rats is 138 mg kg^{-1}). The ANIC plant management only admitted the risk to local residents when the results of tests carried out by health officials confirmed a serious pollution hazard. The mayor of a town in the affected area appealed to the Italian government for assistance; no response came for several days. ANIC officials now say all but 1 km^2 of the area has been rendered harmless.

This has been done by spraying with calcium chloride and ferric sulphate solutions. When arsenious oxide is in a soluble form these two additives will form complexes of calcium pyroarsenite and ferric enneaoxyarsenite respectively. Both complexes are considerably less soluble than the oxide and would thus reduce the amount of arsenic leaching into the water system and spreading far beyond the present area of pollution.



The cost mounts

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USA

Nuclear power's litmus test

A crucial test of public confidence in nuclear power will take place in six states during next month's Presidential election. Colin Norman reports from Washington.

WHEN residents of Arizona, Colorado, Montana, Ohio, Oregon and Washington mark their ballot papers on November 2, they will do more than cast votes for candidates for sundry political offices. They will also vote on resolutions which would place strict—some say crippling—controls on nuclear power plants in their states.

The resolutions are stirring up considerable debate, and large sums of money are being spent both to promote and defeat them. Although the number of power plants affected is relatively small compared with the total number planned for the United States, the votes are regarded as a litmus test of public attitudes towards nuclear power. With the election less than a month away, opinion polls indicate that the resolutions stand a good chance of being approved in at least two states—Colorado and Oregon.

Although there are some differences between the resolutions on the ballots in the six states, they would all set three important conditions on the operation of nuclear power plants.

- There must be no limit to the total amount of damages which could be claimed by victims of a nuclear accident. At present, a federal law, the Price-Anderson Act, limits the nuclear industry's liability to a total of \$560 million for each accident.

- Before new power plants could be operated, the state legislature would have to be convinced that major safety systems—including the much-discussed emergency core cooling system—would operate properly in an emergency. Approval would require a two-thirds vote in the legislature.

- Similarly, at least two thirds of the legislature would have to be satisfied that adequate, proven technologies exist for transport, storage, and disposal of radioactive wastes.

At present, it is doubtful that the nuclear industry could meet those conditions. Thus, passage of the resolution in any state would virtually rule out nuclear expansion in that state.

Voters in California were presented with a similar choice last June, when a nuclear safety proposition was placed on the ballot papers in the Presidential primary elections. That proposition, which would have imposed the same three conditions on nuclear plants in

California, was eventually defeated.

Supporters of the resolution in Oregon and Colorado, and to some extent in the other four states, expect to fare better, however. For one thing, the steam was taken out of the California campaign at the last moment when the state legislature passed a package of nuclear safety bills which included some of the proposition's requirements. But, more important, the California proposition would have applied to existing, as well as planned, power plants. The Oregon and Colorado measures, by contrast, would specifically exempt existing plants.

A potentially important factor in the vote on the Oregon resolution is that during the Oregon primary election earlier this year, Jimmy Carter, the Democratic Presidential candidate, virtually endorsed the resolution. He said that although he would not try to advise people how to vote on the matter, if he were an Oregonian, he would support the resolution. He did not endorse the California proposition, however, largely because it would have

applied to existing plants.

Supporters of the resolution also like to point out that both Colorado and Oregon have approved a number of environmental laws long before other states. Oregon, for example, was the first state to outlaw the sale of plastic bottles and aerosol sprays containing fluorocarbons, and among Colorado's environmental achievements is the passage of a resolution which stopped the Winter Olympics being held there.

But the most solid piece of evidence that the nuclear industry is in trouble in Colorado and Oregon comes from public opinion polls published early in October in newspapers in Denver and Portland. They suggested that public sympathy was then running nearly two to one in favour of the resolutions. Although that margin is sure to close as the election draws near industry officials say they are very concerned.

Thus, if the resolutions are adopted by any state this November, it would give the anti-nuclear movement a boost in future efforts. But, by the same token, if the resolutions are defeated, the nuclear industry could claim a vote of confidence. Clearly, the stakes are high. □

GAO knocks ERDA

Although the Energy Research and Development Administration (ERDA) is responsible for developing nuclear energy technologies in the United States, ERDA officials insist that they have not tried to influence the outcome of the referenda on nuclear safety being held in various states. Those assertions are, however, called into question by a report published last week by the General Accounting Office (GAO), an investigative agency of the US Congress.

According to the report, between February and April 1976—when debate on the California referendum was raging—ERDA distributed 78,600 copies of a pro-nuclear pamphlet in California. According to GAO, the pamphlet “was not objective, is propaganda, and was not a proper document for release to the public”. The pamphlet was used by the nuclear industry in California in their campaign to defeat the California proposition.

ERDA officials have claimed that the document—a series of so-called myths and facts about nuclear power—was prepared solely for internal distribution within the agency. It was intended, they have argued, for employees in the controversial liquid metal fast breeder reactor pro-

gramme, to help answer criticisms of the programme and to lift morale.

GAO found such assertions hard to swallow, however, since the fast breeder programme employs only about 6,700 people, yet 100,000 copies of the pamphlet were printed. Moreover, GAO found it difficult to understand why the bulk of the copies were sent to California, while most of the breeder reactor work is going on in other states.

Nevertheless, GAO stopped short of actually accusing ERDA officials of trying to influence the California vote—in fact, it specifically states that it could find no such evidence and suggested that ERDA officials had not actually violated any laws. The report did not attempt to explain why so many copies of the pamphlet were distributed in California at that particular moment, however.

As for the quality of the pamphlet, the GAO report suggested that it was so superficial and misleading that it was not even suitable for distribution to ERDA employees as part of morale lifting effort. “ERDA should not place itself in the position of misleading others—whether it be the public or its own or contractor employees—for the sake of raising morale”, the report argued.

CANADA

A matter of judgment

David Spurgeon writes from Ottawa on a report covering Canada's problematic science-industry relationship

IN 1973, the Ministry of State for Science and Technology (MOSST) introduced a "Make-or-Buy" policy, the aim of which was to increase the proportion of government-funded research and development (R&D) carried out under contract by industry as opposed to government laboratories. The government hoped to strengthen the innovative capacity of Canadian industry and to enhance its competitive position. The policy has not generally been regarded as a howling success, especially by industrialists. In the year following its introduction, in fact, the Cabinet found it necessary to expand the original policy to allow financing of unsolicited proposals for R&D from the private sector.

A steady decline in support has generally been seen in the government's R&D funding policies. A brief from the Royal Society of Canada to the Prime Minister said that, although it was generally recognised in 1969 that government support for industrial R&D was already too small, it is now even smaller: in terms of the fraction of federal budgetary expenditures it had fallen by 1975 to 68% of the 1969 level.

Now a report has come from the MOSST* conveying the message that the Make-or-Buy Policy has in fact been rather successful, and that more of it is needed. The report is full of statistics supporting its argument, but its general tone appeared so contrary to what could be heard in non-government quarters that it left the impression of being a rather curious document.

A little investigation suggests why: the choice of statistics is highly selective, some say misleading. In fact it is questionable in some cases whether they show what they are purported to show. One table, for example, shows the increase since 1973-74 of mission-oriented contracts awarded to industry by the Department of Supply and Services on behalf of the various government departments, the intent being to indicate that they have increased. Yet the table lumps together all contracts from government departments, as though all resulted from the policy. In fact government departments have contracted out to industry for years, to a greater or lesser degree.

*The Make-or-Buy Policy 1973-75 (Industry Branch, Ministry of State for Science and Technology; November 1975)

One diagram showing payments to Canadian industry for R&D between 1963-64 and 1975-76 shows not only that research contracts have increased since the Make-or-Buy policy was initiated, but also that, years before, in 1965-66, such contracts were larger than any year until 1973-74, indicating that the grants level fluctuates from year to year, and that taking just a short-term view (that is, from 1973-76) can be misleading. (The year 1965-66 was a good one largely as a result of the hydrofoil project of the Department of Defense.)

Analysis of the figures used in the report actually shows that government R&D contracts to industry had already begun to rise *before* the Make-or-Buy policy was put into effect, and, plotting them on a graph, that the level of R&D contracts to industry would be at the same point they reached even if they had merely been extrapolated at their former rate.

Furthermore, the ratio of payments to industry vs. intramural R&D was less in 1975-76 than a decade earlier by a substantial margin (even though that was only for a single year). Even since the Make-or-Buy policy was introduced, the increase in industrial contracts has amounted to only roughly \$20 million, while the increase in in-

house research funds has amounted to \$57 million. And in terms of overall science expenditures by the federal government, industry's share has fallen from 19% to 16% during the three-year period of the policy.

The report, which was only released this summer, admits that it is premature to assess whether the ultimate economic effect aimed for in the policy is yet being achieved. But it does claim that the policy has had a beneficial effect on several sectors of industry. At the same time, it admits that during the course of the past five years, the proportion of Canada's total resources directed at R&D has steadily declined: gross national expenditure on research and development as a percentage of GNP had dropped in 1972 to 1.14% from 1.29% in 1969, while the Senate special committee on science policy had said it should be about 2.5% to keep Canada competitive internationally.

The report finds that the effects of the policy to date have been most pronounced in electronics, transportation and scientific services, where objectives of government and industry most closely coincide in the context of current departmental missions. If the programme were to be extended—which the report thinks advisable—either a reduction in government scientific staff or a substantial increase in funds for contracts would be necessary. □

Trudeau rings the changes

Stung by the results of a public opinion poll that showed Canada's Liberal Government to be lagging far behind the opposition in popularity, and by criticisms of both his bilingualism and anti-inflation programmes, the Canadian Prime Minister, Pierre Trudeau, recently announced wide-ranging Cabinet changes that have produced a new head for the Ministry of State for Science and Technology (MOSST).

Gone not just from the ministry, whose portfolio he held jointly with that of Public Works, but also from the government itself, is C. M. "Bud" Drury, who at 64 was one of the two most senior Cabinet members (with Mitchell Sharp, who has also resigned). In his place is Hugh Faulkner, a more junior minister who, according to the *Toronto Globe and Mail's* Ottawa correspondent, "was demoted from Secretary of State to Minister of State for Science and Technology mostly because the Liberal caucus felt that he had failed to sell bilingualism, a special responsibility of the Secretary of State."

Be that as it may, the new appointment is not calculated to cause rejoicing throughout the science community. Mr Faulkner's appointment makes it obvious that the science ministry will continue to be regarded by the government as a low priority, as it has been from its inception in 1971. Apart from Mr Drury, Mr Trudeau has always appointed inexperienced or junior ministers for this post.

The new appointment comes as just one more disappointment for the science community. But to many, Mr Drury's attitude towards the needs of science in Canada had in recent months seemed apathetic if not openly hostile. In spite of studies and recommendations on national science organisation, the government in general and Mr Trudeau in particular apparently lost interest in science and technology. The concerns of bilingualism and inflation had loomed so large as to eclipse science, and doubts had grown about the government's professed concern for the problems of science-based industry.

IN BRIEF

ESA moves

The European Space Agency's Scientific Programme Committee has approved the spending of about \$115 million on two projects: a contribution to NASA's space telescope, due for launching in 1983, and the Geosari project involving the Geos spacecraft and Ariane launcher.

The \$104 million provisionally allocated to the space telescope will provide 15% of the costs of the telescope and its associated Science Institute. In exchange European astronomers will get at least 15% of total observing time.

Negotiations with NASA on another ESA/NASA cooperative project, the Out-of-Ecliptic Mission, will continue. LIRTS (Large Infrared Telescope on Spacelab) and several other projects will be considered in the spring.

The council of the European Space Agency has appointed a new director of the Spacelab programme, M Michel Bignier. Until June 1976 M Bignier was Director General of the French Centre National D'Etudes Spatiales.

MRC report warning

The UK Medical Research Council (MRC), referring to the dual-support system for research in its annual report published this week, warns that, under extreme pressure, it would be contractually obliged to protect the security of its own 4,373 staff at its 68 research establishments, and the capital investment associated therein, if necessary at the expense of its indirect support for research at universities. Such a situation, the outgoing Secretary Sir John Gray emphasised, was not yet foreshadowed, and MRC policy remained one of parity between the two lines of support.

Government departments have re-deployed with the MRC the 25% of the council's grant-in-aid from the Treasury transferred to them under the Rothschild arrangements. Total expenditure for the year amounted to £47.18 million, up 30%, of which international subscriptions, which grew 66%, were £960,000. Increases in these subscriptions because of exchange rate variations, along with pay and asso-

ciated awards, made three additions to the grant-in-aid necessary. The current doctrine of cash limits, said Sir John, meant that any extra money to meet international obligations would have to come from ongoing work.

SGHWR inquiry continues

Following the summer recess, the UK House of Commons Select Committee for Science and Technology has resumed its inquiry into the decision surrounding Britain's involvement with the SGHW reactor with evidence from the Central Electricity Generating Board (CEGB) last week and from the United Kingdom Atomic Energy Authority (UKAEA) earlier this week.

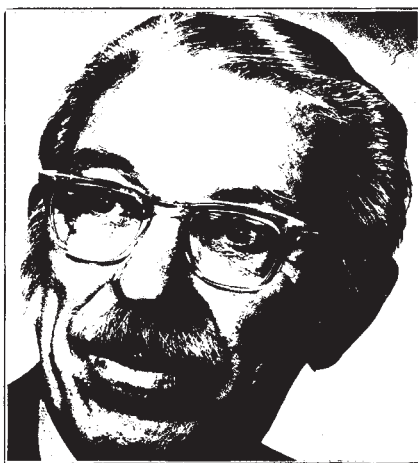
In another development, a report from a study group chaired by Dr Walter Marshall on the integrity of pressure vessels for light water reactors was published by the UKAEA last week. The 156-page report makes 40 "essential recommendations" and has buttressed the case for PWRs and against SGHWRs in Britain.

THE pertinacity of the promoters of laetrile for cancer "cure" is probably related to the amount of funds at their disposal. Some possible clues are in a grand jury indictment in California, stating that 10 ml of laetrile, sufficient for three daily injections, cost American cancer patients as much as \$60 and that a physician who administers laetrile "as a vitamin supplement" deposited \$2.5 million in northern California bank accounts between May 1974 and August 1975. The president of the Committee for Freedom of Choice of Cancer Therapy was arrested last December with \$40,000 worth of laetrile in his car, along with a loaded Browning automatic pistol. Previous cancer remedies such as Krebiozen and the Hoxsey remedy were on a smaller scale. The big question is: will political pressure, financed by profits from laetrile, overcome the opposition of organised medicine to its legalisation?

The Merck Index describes laetrile as the β -glucuronide rather than the β -glucoside, of L-mandelonitrile. It seems probable, however, that the laetrile of "underground commerce" is actually amygdalin (the β -glucoside). An argument used by the pushers of laetrile is that it is "vitamin B₁₇". In support of this, one of them stated that "it becomes almost impossible, on the negative side ever to declare scientifically that a compound is not a vitamin...". Constance Holden, a

staff writer for *Science*, echoing this statement, said on September 10, that "no one has really been able to prove" that there is no such vitamin (as B₁₇). She also said that it is a "fact that scientists are not able to defini-

Laetrile struggles



THOMAS H. JUKES

tively refute the nutritional claims about laetrile...". Actually nutritionists feel no inhibitions about refuting such claims.

The cyanogenic glycosides, including amygdalin, have no resemblance to vitamins. The accepted definition of a vitamin is that it is necessary in the diet of at least one vertebrate organism to prevent a

specific deficiency disease. The Committee on Nomenclature of the American Institute of Nutrition found no scientific evidence (i) for the existence of a nutrient identified as vitamin B₁₇ or (ii) that laetrile has nutrient properties or nutritional value for "either animals or humans" (*sic*). Cyanogenic glycosides are often poisonous to livestock. One of them, linamarin, is present in cassava, and sometimes causes human blindness.

The persistent efforts of laetrile proponents were successful in Alaska where, on June 21, 1976, Governor Hammond allowed the "Laetrile Bill" to become law, thus permitting physicians to prescribe laetrile. His excuse was an implementation of the "equal time for nonsense" principle, which says that if there are two sides to an issue, it is not the role of a statesman to decide which one is right. The history of scientific medicine as applied to public health shows that it is insufficient to make discoveries that benefit human beings. It is equally important to get rid of quack remedies to make room for new advances. Until recently, legislators accepted informed advice in framing public health laws, but Alaska is "different".

The latest news is fascinating: the Assistant Attorney General of Alaska now says that sale or distribution of laetrile in Alaska by any person, including a physician, is illegal. There may be a problem in filling those prescriptions.

correspondence

Future of journals

SIR,—Your editorial of August 26 (p. 731) on the future of journals is typical of the current negative attitude to the problem. It is a pity that the more constructive contribution on the subject from Professor May and his wife (*Nature*, February 12, p. 446) was not mentioned.

The rising costs of running a library are eating into the money available for the purchase of publications, and yet we constantly read that there are too many journals, never too many librarians. The main library is often rightly the showpiece of the modern university campus, but are all librarians aware of their primary responsibility? Rows of expensively bound journals may satisfy the eye, but a drawer or binder of microfiche could be more relevant to academic needs.

The "pious hope" that two journals might be shut down for every new one launched would not be considered particularly pious by the users of the two guillotined journals. It would be more sensible in the short term to cut down on costs on both sides, amalgamate smaller, ailing periodicals and employ new methods such as those pioneered by the chemical societies in the United States and Britain.

We gather from various journal publishers that despite the economic conditions prevailing in many countries, reasonably priced research journals of medium circulations (1,000–4,000 subscribers) have generally enjoyed a steady increase in circulation, which has been maintained this year. This is an inevitable result of the worldwide growth of the academic community, and possibly also of the rationalisation of library purchases; for example, a university which may take 10 subscriptions to a major journal, or an 'essential title', for various departments, might cut down to two for the main libraries (*Nature* beware!), thus releasing funds for new acquisitions.

The long-term result of this trend might well be a huge number of journals distributed to, say, 1,000 resource/information centres scattered around the world. Researchers would then obtain photocopies of original papers, having browsed through synopses and abstracts, at their local library, by then reduced to little more than a post-office. This surely could lead to delays of a week or more. Faced

with such a possible outcome perhaps researchers and librarians might learn to love cabinets of microfiche.

May we therefore suggest that the present number of 35,000 journal titles should be viewed as a challenge, and not approached with despair. The final suggestion in your editorial, that authors should supply full details on request, would be a long step backwards. Imagine trying to trace an author from his last known address; with the present postal services it could take months. Who would be prepared to referee papers which might never be read? What use would data lists be in job applications? Evidence could be altered at a later date as no dated record would exist. Such chaos should be avoided. More organisation is required, not less.

PETER ASHBY

ROBERT CAMPBELL

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SIR,—I should like to comment on your editorial about the future of journals (August 26). As librarian in a research laboratory, I would agree with most of what you say—with the proviso that the changes you refer to should be reflected not only in journal subscription price, but in volume of paper. This is conspicuously not the case in the experiment of the American Chemical Society, where the weight of paper is much higher in the experimental than in the traditional journal.

As a practising organic chemist, I must, however, reply a firm "no" to your last question. Chemistry in general, and organic chemistry in particular, is an experimental science, and we must have the experimental detail readily available. One of the greatest weaknesses of the *Bulletin de la Société Chimique de France* was that experimental sections were abbreviated to the point of irreproducibility, although things have improved recently. There is a large body of opinion (perhaps even a majority) which recognises that theoretical sections are usually fitted to experimental sections after the event; and indeed, what some would like to see is a journal in which only the experimental section and the literature list is printed, the theoretical section being available on microfiche if the reader were unable to supply his own!

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Mistaken impression

SIR—I fear that Wil Lepkowski, in quoting from a paper that I presented at the Conference on Tradition and Change in Physics Graduate Education at Pennsylvania State University in August 1974, may have inadvertently given a mistaken impression of my position (August 12, page 528).

As I state in the same paper, "The Berkeley Group missed out on making these discoveries not so much in spite of the fact that their laboratory was so large, efficient, well-run and well-managed as precisely because of it".

The purpose of my paper was not to criticise a particular laboratory, but to argue, on the basis of historical experience, that the techniques of "Big Science", while they may be useful in solving certain engineering problems, as in the case of the Manhattan Project or the Apollo Program, are by their very nature not conducive to the making of fundamental, new scientific discoveries. Condensations of my paper were published in *New Scientist*, 63, 462 (1974) and *Center Magazine*, 55 (May–June 1975).

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Biological fly killer?

SIR,—The news on Human Trypanosomiasis Today by F. E. G. Cox (*Nature*, August 19, p. 646) calls for some views. As long as the immunological control of sleeping sickness remains unlikely, eradication of the genus *Glossinia* seems to be the only way to control the disease. Cox cited recent successes in Nigeria where a riverine area of 27,500 km² was sprayed aerially. The same technique was recently used over parts of Bangladesh in an attempt to control mosquitoes. The disappearance of quite a number of insect species was subsequently noticed, though all of them may well have made a come back, and for sure the mosquitoes did. Bearing in mind the delicacy of ecological balances nobody should be satisfied with aerial spraying as a method of controlling insect species. Intensive efforts should be mounted to introduce methods of biological control, such as releasing specific pathogens.

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news and views

Non-histone proteins and transcription

from Carol K. Klukas

THE genomes of mouse and of humans, having molecular weights of 3×10^{12} daltons each, contain enough DNA to code for thousands of genes. Some of these sequences are present in multiple copy form while others are included only once; some are transcribed in all cells and their presence is essential for proper cell function while others code for proteins of more limited function and are transcribed only in certain specialised cells, or perhaps only at specific times in the development of an organism or even only at specific phases in the cell cycle; some sequences code for proteins which eventually must be produced in very large quantities in a cell while other sequences will dictate the synthesis of only a few protein molecules. How is it that all the sequences can be sorted out and be transcribed or not transcribed in just the right quantitative and temporal manner to result in proper function of a cell and of an organism? What factors are involved in the control of eukaryotic genome expression?

Since the answer to this complex question must lie at least in part in the composition and structure of chromatin (the DNA with the histones and non-histone proteins which are complexed with it *in vivo*), much current research is designed to answer various specific questions concerning chromatin. Three papers published back to back in the July issue of *Biochemistry* are noteworthy examples of such research.

In each of these papers, RNA transcription *in vitro* from isolated chromatin is compared on several criteria with *in vivo* transcription to determine whether *in vitro* transcription is a faithful representation of transcription as it occurs in the cell. Once this has been established, the composition of chromatin can be changed in a controlled way and the resultant changes in transcription can be analysed. This approach should reveal which aspects of chromatin composition and organisation are essential for the maintenance of *in vivo*-like transcription.

In the first of the papers, Bacheler and Smith (*Biochemistry*, **15**, 3281; 1976) compare total *in vitro* synthesised transcripts of mouse liver chromatin with nuclear RNA by means of RNA excess hybridisations with small amounts of radioactive mouse DNA which has been fractionated on hydroxyapatite into three classes each differing from the others in the reiteration frequency of the sequences it contains. The results of these experiments indicate that all sequences found in nuclear RNA are also transcribed *in vitro*. Furthermore, the transcription of reiterated sequences from isolated chromatin is restricted in just the same way as is transcription *in vivo*, that is, highly repetitious sequences in mouse DNA are not represented in either nuclear RNA or in RNA transcribed from chromatin *in vitro*. Competition experiments show that there are sequences transcribed *in vitro* from chromatin which are not present in nuclear RNA. But these belong to a very limited set of sequences unless the chromatin is extracted with 0.5 M NaCl before transcription. This salt extraction, which strips the chromatin of H1 histones as well as of certain non-histone chromosomal proteins, results in transcription which is no longer sequence restricted. Thus, Bacheler and Smith show that at least some significant aspects of *in vivo* transcription can be preserved *in vitro* and can be altered by salt extraction, suggesting the importance of H1 histone and/or of some of the acidic chromosomal proteins in transcriptional control.

Quite a different way of analysing *in vitro* transcripts, which is used in the other two papers (pages 3291 and 3296), is to assay for the presence of transcripts of one specific gene or set of genes, in this case the histone mRNAs. Stein, Reed and Stein have prepared ^3H -cDNA complementary to histone mRNA and used it in hybridisation experiments to probe for histone mRNA in transcripts from various reconstituted DNA-chromatin complexes prepared *in vitro* from fraction-

ated HeLa cell chromatin. These studies show that naked DNA is an effective template for *in vitro* transcription of histone genes (see also Huang *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **48**, 1216; 1962; Allfrey *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **49**, 414; 1963) but that this transcription is inhibited nonspecifically by the addition of histones. Further, non-histone chromosomal proteins mixed with DNA in the *in vitro* system have no effect at all on the rate of histone gene transcription or on the frequency of histone mRNA in the transcripts unless however the non-histone proteins are mixed with the DNA in the presence of histones. In this last instance histone mRNA genes are made selectively transcribable, and thus histone mRNA represents a higher percentage of the total transcript. This result indicates that the interaction of histones and non-histone chromosomal proteins must be involved in a significant way in the control of specific gene transcription in this system and as the same effect of non-histone proteins has been observed in globin gene transcription (Paul *et al.*, *Cold Spring Harbor Symp. quant. Biol.*, **38**, 885; 1973; Barrett *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 5057; 1974; Chiu *et al.*, *J. biol. Chem.*, **250**, 9431; 1975) it is quite likely that histone-non-histone protein interaction is a general transcriptional control mechanism.

In these experiments the chromatin used was isolated from HeLa cells in the S phase of the cell cycle. In the last paper (page 3296) Park, Stein, and Stein investigate the *in vitro* transcription of G₁-phase chromatin and report that they find very little histone mRNA in the transcripts. But if S-phase chromosomal proteins are added to G₁-phase chromatin, the specific transcription of histone genes is enhanced. When G₁ and S-phase chromatins are fractionated and reconstituted using combinations of pooled DNA and pooled histones with varying amounts of G₁ or S-phase non-histone proteins, it becomes clear that S-phase chroma-

tin contains a non-histone protein which has the ability to render the histone genes available for transcription. It is the absence of the protein in G₁-phase chromatin rather than the presence of some inhibitor molecule which distinguishes G₁-phase from S-phase chromatin. Thus the importance of the non-histone proteins and their interaction with DNA and histones in the control of specific genes is again indicated.

The type of *in vitro* transcription and reconstitution experiments presented in these papers are beset with difficulties not only in their execution, but also in their interpretation. Often such work meets with much criticism and scepticism because factors such as just how the DNA probe was made and how the reconstitution and hybridisations were performed can alter the results significantly and consequently lead to misinterpretations. Also one can always criticise this type of work by pointing out that the studies are after all *in vitro* and may have nothing to do with the transcriptional process which occurs in the cell. Certainly this is correct; however, *in vitro* transcription and reconstitution experiments despite their difficulties are useful in pointing out which components of the *in vivo* system may be of particular significance. In a system as complex as that for the control of eukaryotic genome expression, such clues to which factors are likely to be most important is certainly useful as a guide for further thought and research. □

Higher ozone concentrations over Britain

from Peter D. Moore

THE elevated levels of ozone in the atmosphere of London and other parts of Britain have been a source of concern for some years (see *Nature*, **256**, 537; 1975). Man-made pollution is undoubtedly a major source of ozone in the troposphere and inflates the 2-4 p.p.h.m. which is normally found there. Petrol and diesel engines are considered to be important in this process of ozone generation, for they emit nitric oxide and also uncombusted hydrocarbons which oxidize the nitric oxide to nitrogen dioxide. NO₂ is liable to photochemical dissociation in daylight leading to the generation of atomic oxygen which interacts with molecular oxygen to produce ozone, O₃.

Ozone is a powerful oxidant and is known to damage both animal and plant tissues (Bell and Cox, *Environ. Pollut.*, **8**, 163; 1975). The Environmental Protection Agency of the United States has established an air quality standard of a maximum ozone concentration of 8 p.p.h.m. for an hour's duration. In London, the Greater London Council's guideline has been set at the same level.

For some years this guideline has been exceeded, usually during anticyclonic periods in summer. On July 13, 1972, a peak value of 11 p.p.h.m. O₃ was recorded in Central London (Derwent and Stewart, *Nature*, **241**, 342; 1973). On page 580 of this issue of *Nature* Ball presents data for the summer of 1975 in London and registers a peak value of 15 p.p.h.m. on June 26, 1975, almost twice the GLC guideline level. This is the kind of concentration at which, according to the WHO and the US Department of Health, a degree of eye irritation and aggravation of respiratory diseases may be expected.

This is still well below the 28 p.p.h.m. of Sydney, over 30 p.p.h.m. in Tokyo and up to 99 p.p.h.m. in Los Angeles. It is, however, a disturbing trend. Ball has kindly provided some preliminary figures for the current (1976) summer in London which suggest that the trend towards higher ozone concentrations in Britain is continuing. Even in early May of this year, levels of 18 p.p.h.m. had been recorded in London. Subsequently values up to 21 p.p.h.m. have occurred, and the guideline concentration has often been exceeded for as many as 40 h per week over the whole of London. Records from Harwell are even higher than these.

Ball's data do help to resolve the question of whether Britain's ozone is largely derived from the Continent or whether our cities make a significant contribution. Cox *et al.* (*Nature*, **255**, 118; 1975) showed that photochemically generated ozone can be transported distances of the order of 100-1,000 km. Continental ozone can enter the British troposphere in sufficient concentration to raise ambient levels to as much as 15 p.p.h.m., as was shown during easterly air movements at a recording station in Suffolk during August 1973. Stewart *et al.* (also in this issue of *Nature*, page 582) show that high ozone concentrations occur most often on days when the wind direction is north-east, through to south. This lends some support to the contention of Cox *et al.*, but it is also true that when anticyclonic conditions prevail the wind is most often in that quarter. As Stewart shows, ozone is highest when

temperature and insolation are high, and these conditions are also associated with anticyclones. Ball now demonstrates that among the London recording stations higher values of ozone are found at those sites which receive air masses from over Greater London, and concludes that home-grown ozone represents a significant contribution to our tropospheric load.

To what extent are these recent high levels of ozone a consequence of the unusually sunny, anticyclonic summers we have experienced of late? Only continued monitoring of gaseous pollution over London can answer this. It is salutary to note that in cities like Sydney, ozone became a problem with startling suddenness. A further, unanswered question is the degree to which ozone interacts with sulphur dioxide, our other main gaseous pollutant, in its harmful effects. Menser and Heggstad (*Science*, **153**, 424; 1966) showed that tobacco plants were severely injured by mixtures of SO₂ and O₃ in concentrations of 24 p.p.h.m. and 3 p.p.h.m. respectively, whereas neither of these concentrations produced damage on their own. It is possible that this synergistic effect is general, in which case ozone may be an even more serious pollutant than is currently predicted, especially in cities such as London where SO₂ concentrations are still high. □

Pleistocene mammals

from D. W. Yalden

Historically, the evidence provided by fossil mammals played a large part in establishing the glacial theory of Pleistocene geology. The discovery in the 1860s of fossil lemmings in southern England, in particular, was readily accepted as evidence of former tundra conditions. Though early excavations of caves and river terraces concentrated on large mammals, such as mammoths, or on human remains and artefacts, much information on smaller mammals was obtained; this formed the basis as long ago as 1926 for a large (though uncompleted) monograph by Hinton (*Monograph of Voles and Lemmings (Microtinae) Living and Extinct*, British Museum, (Natural History), London). While this early work established Quaternary biology as a science, it suffered, as pioneering work inevitably must, from being too early. The fossil mammals were subjected to an extremely typological classification, with names founded on extreme variants, and more normal intermediates left unnamed. The very complex nature of

ALTHOUGH it was the first nuclear process studied by Rutherford, alpha decay is still not properly understood. A large number of alpha decay rates have now been measured for heavy nuclei, and these depend basically on two factors, a nuclear structure factor that gives the probability that there is an alpha particle in the nuclear surface 'waiting to get out', and a penetration factor that gives the probability that it will be able to tunnel quantum-mechanically through the barrier and so escape from the nucleus.

Ever since Gamow first applied quantum mechanics to the nucleus, it has been known that the penetration probability depends very critically on the height and width of the potential barrier through which the alpha particle has to tunnel, and hence on the energy of the emitted alpha particle. This is why the alpha decay half-lives vary from a very small fraction of a second to many thousands of years, while the corresponding alpha energies vary by less than an order of magnitude.

This simple theory was very successful in accounting for the relative alpha decay rates of a large number of radioactive nuclei, but the absolute values were much less certain, due to the lack of knowledge of the nuclear

structure and of the potential barrier. Using the best available nuclear structure information, there was usually a factor of a thousand between the measured and calculated decay rates and it was generally considered that this large factor was still within the uncertainties of the potentials.

This point has now been carefully checked by De Vries at Rochester and Lilley and Franey at Minnesota (*Phys. Rev. Lett.*, **37**, 481; 1976). They

Absolute alpha decay rates

from P. E. Hodgson

studied the interaction of alpha particles with ^{208}Pb and ^{209}Bi and fitted the experimental data for elastic scattering at 22 MeV and the reaction cross section from 17 to 24 MeV by a series of optical model potentials. The reaction cross sections are known accurately since they are the sum of the (α, n) , $(\alpha, 2n)$ and (α, γ) cross sections, all of which can be measured accurately. No other reactions are energetically possible.

They found that this data imposes tight restrictions on the optical

potentials, so that an acceptable fit can only be obtained with parameters constrained within narrow limits. The penetrabilities calculated from these potentials vary somewhat, but far less than was previously thought. This makes it possible to calculate accurate absolute values of the quantity called the reduced alpha width γ^2_α that contain the nuclear structure information alone. It is found that these values are still about a thousand times greater than those calculated from the shell model. For example, for ^{210}Po , the value of γ^2_α found from the measured alpha decay rate and the calculated penetrability is $0.88^{+4.4}_{-0.19}$, whereas the value calculated from the shell model by Harada is only $(0.31 \text{ to } 3.3) \times 10^{-3}$. Results for other nuclei are similar.

This is a serious discrepancy and shows the need for much more sophisticated nuclear structure calculations, including more configuration mixing and the renormalisation required by antisymmetrisation. A thousand times more alpha particles are emitted than would be expected from existing shell model calculations, so it may well be that this is due to substantial clustering of alpha particles on the nuclear surface that cannot yet be described by the shell model. □

the climatic fluctuations was not appreciated either (indeed Hinton was a monoglacialisist, and so assigned faunas either to "the" cold period or to the preceding warm period), with the result that much ecological and stratigraphical information was overlooked or lost.

Biological aspects of Pleistocene study have been revolutionised first by pollen analysis and more recently by the study of insect, especially beetle, faunas; physical aspects of study have added absolute chronologies (from radiocarbon and other dating techniques) and absolute temperature calibration (from oxygen isotope ratios). In this surge of knowledge, Pleistocene mammals have, at least in Britain, been left far behind; Zeuner's book (*The Pleistocene Period*, Hutchinson, 1944) contained a chapter of 30 pages on mammals, but only four single-line entries on pollen analysis, whereas West (*Pleistocene Geology and Biology*, Longman, 1968), has 32 pages on pollen stratigraphy but only six on mammals.

Clearly the time was ripe for restudy of British Pleistocene mammals, not only in their own right but also to integrate them into the newer framework of Quaternary chronology. Some significant signs of such restudy are

now available. One is Stuart's review relating all available vertebrate occurrences to the pollen stratigraphy of Britain (*Biol. Rev.*, **49**, 225-266; 1974), this involved new collecting from sites where information on pollen was available or obtainable as well as a re-examination of a scattered and often obsolete literature. Another equally valuable review of a different type is Maglio's paper on the evolution of the elephants (*Trans. Am. phil. Soc.*, **63**, 1-149; 1973). Because they are so conspicuous, fossil elephants, or at least their molars, have played an important part in stratigraphy, but their taxonomy has been extremely muddled.

Perhaps the most important such contribution has just been published by Sutcliffe and Kowalski (*Pleistocene Rodents of the British Isles*, *Bull. Br. Mus. (nat. Hist.) Geol.* **27**, 31-147; 1976). This account is important in a number of respects. For a start, the rodents are, and have been throughout the Pleistocene, the most numerous mammals; they are therefore the most abundant in cave deposits, and so potentially the most useful archaeologically or stratigraphically, and they are arguably the most important ecologically. Their potential has however been largely unrealised, partly perhaps be-

cause archaeologists have tended to ignore them, but largely because, as a result of the early studies, they were in a taxonomically unsatisfactory state and therefore unreliable stratigraphically. This review should at least resolve these last obstructions. The literature records are thoroughly reviewed, and their stratigraphical assignments reassessed. The taxonomy is also reviewed, so that it is now much clearer just how many distinct species were present in the Pleistocene. The dubious taxa erected by Hinton, among others, are thoroughly scrutinised, and many are relegated to synonymy. Of particular importance is the cautious reassessment of *Microtus* species, especially *M. arvalis*. This vole has a rather southern distribution in Europe, being absent from Scandinavia and, except for Orkney and Guernsey, from Britain. The occurrence of fossil remains attributed to it, however (often as *M. corneri*) have led to suggestions that it was an early post-glacial immigrant, since isolated as a relict in Orkney. Not only is this inherently unlikely on ecological grounds (human introduction being more likely an explanation) but, as this review makes clear, the supposed fossil occurrences are very doubtful indeed. The

review also reveals however many very real problems which require further study or perhaps further material. For example, we do not know for certain when lemmings disappeared from Britain nor whether, as the evidence suggests, the high Arctic collared lemming, *Dicrostonyx torquatus*, survived later than the more southerly *Lemmus lemmus*.

More important, however, than this sort of query which the review poses is the framework which it provides for evolutionary studies. G. R. Coope (personal communication) has averred that beetles, because of their mobility, provide a better indicator of past climates than plants, but that their reliability for this depends on their evident morphological stability and presumed ecological stability throughout the Pleistocene. Plants also seem to have evolved little. The rodents, however, share the mobility of beetles, but at the same time demonstrate considerable evolutionary flexibility. Their rates of evolution and the relationship of these to ecological changes are aspects which should begin to attract attention.

Cosmic rays at Leeds

from a Correspondent

The fifth European Cosmic Ray Symposium was held at Leeds on September 14–17, 1976.

COSMIC ray studies still have a lot to offer the nuclear physicist. That at the very least was clear from the discussions at the symposium. The energies available with cosmic ray particles (up to $\geq 10^{20}$ eV) still far exceed the best accelerators. Thus effects such as high transverse momentum and increasing cross sections for hadron interactions that are perhaps beginning to appear at the highest machine energies should be clearly observable from cosmic ray studies. If, as accelerator work initially seemed to indicate, Feynman scaling theory holds then it should become very evident at these high energies.

Experimentally, problems arise in studying high energy cosmic rays because of their inaccessibility and very low flux. The high energy primary particles reaching the Earth interact in the atmosphere causing a cascade of secondary particles to reach sea-level in the form of an Extensive Air Shower (EAS). Since the low primary flux makes direct collection virtually impossible these EASs are the only convenient means of investigating the nature and properties of the high energy particles.

A major topic at the Leeds symposium was the attempt to relate the experimentally measured properties of the EAS to theoretical predictions based on cascade calculations assuming both a nuclear physics model and a particular mass composition spectrum for the primary particles. High transverse momentum phenomena in nuclear interactions (diverging from average CKP behaviour) have been a topic of discussion at cosmic ray conferences for many years, and now positive evidence is also appearing from accelerators. Controversy still reigns, however, as to the magnitude of the effect or indeed whether the observed events could be just the expected high momentum tail from the CKP distribution.

The scaling theory of nuclear interactions came in for a lot of hammering. Evidence is accumulating from EAS research against 'scaling' being operative at high energies. Here a problem in interpretation is the uncertainty in the mass composition of the primary cosmic ray particle initiating the EAS. Assuming the established composition at low energies ($\sim 80\%$ protons) then 'scaling' appears to be incompatible with experimental EAS data. However if predominantly iron primaries are assumed to occur $\sim 10^{17}$ eV the 'scaling' theory can perhaps be rescued. An additional concern, pointed out at the symposium, was the as yet inadequate treatment in the model calculations of extreme fluctuation effects coupled with the steeply falling primary energy spectrum. Rigorous calculations may well make 'scaling' more acceptable. In passing it should be noted that recent results from Fermilab and Stanford on deep inelastic scattering also show departures from 'scaling'.

The most exciting experimental work in EAS studies discussed at the symposium was reported by K. E. Turver and colleagues from the University of Durham. This group have very carefully measured the Cerenkov radiation produced in the atmosphere by EAS. These observations were recorded at the Haverah Park detector array, North Yorkshire, from EAS of primary energy $>10^{17}$ eV. Although such atmospheric Cerenkov radiation has been investigated previously the advance by Turver's group was achieved by making use of a well-spaced array of eight photomultiplier detectors and correlating their responses. By analysing the pulse shape from the different detectors the researchers claim to be able to pinpoint the EAS development with a considerable degree of accuracy. It is fairly early days in their investigations but the technique looks very promising. The main problem at the North Yorkshire moors array is the available operation time as clear dark

nights are required—only 100 hours was achieved last winter. However, if this technique is established at Haverah Park, in close collaboration with the particle detector array there, then it should be possible to set up such an air Cerenkov array independently in a more favourable climate.

The origin and mass composition of the high energy primary cosmic rays still remain the key astrophysical questions to be answered. The extragalactic *versus* galactic origin controversy continues with almost equal support on both sides. Until recently it seemed that cosmic rays even at the highest energies appeared to reach the Earth equally from all directions. However, as the statistics of the really high energy events ($\geq 10^{18}$ eV) built up in recent years there does appear to be some evidence for a small degree of anisotropy in right ascension. A. M. T. Pollock and A. A. Watson (University of Leeds) presented data on the arrival direction of primary particles of energy 10^{17} – 10^{18} eV. Some 45,000 EASs were subjected to harmonic analysis in right ascension. A significant amplitude (0.1% chance probability) occurs in the direction of $251(\pm 15)^\circ$ RA. Evidence from the same group at higher energies ($>10^{18}$ eV) is not so conclusive. There seems to be a change of phase with increasing energy but it is regarded as significant that the two highest energy events ($\geq 10^{20}$ eV) come from close to the North Galactic Pole. D. D. Krasilnikov (Institute of Cosmophysical Research, Yakutsk, USSR) has plotted the arrival directions of the total world accumulation of 67 reported events greater than 4×10^{18} eV. An equatorial region in the galactic coordinate system seems to be practically devoid of events. Krasilnikov concludes that this can be interpreted as the result of screening by magnetic fields of an isotropic flux of the particles coming from outside the Galaxy radiodisk. If these particles were of galactic origin they would be required to be heavy nuclei from a powerful source in the centre or behind the centre of the Galaxy and influenced by a highly extensive radio halo with a very specific regular magnetic field structure. It is more likely that they are of extragalactic origin. Attempts to correlate arrival directions with pulsars have proved negative.

This symposium represented a special occasion in that for the first time the lower energy cosmic ray work (modulation section) was discussed at the same meeting as the high energy and EAS work. Usually two separate symposia are held at different venues. The amalgamation was in honour of Professor J. G. Wilson whose life-long interest in cosmic rays has spanned both branches of the research field. John Wilson is this year retiring from

the Cavendish Chair of Physics at the University of Leeds which he has held since 1952. His research in cosmic rays started under C. T. R. Wilson and he became a prominent member of P. M. S. Blackett's group at Manchester after the Second World War. Latterly he has been responsible for setting up and developing the EAS detector array at Haverah Park. He plans to continue his interest in the field during his retirement.

J. G. Wilson presented a paper dealing with the energy spectrum of cosmic rays at energies $\geq 10^{17}$ eV. Evidence continues to mount in support of a flattening of the spectrum above 10^{19} eV. There appears to be no cut-off at this energy arising, as predicted, from interaction with the supposed universal 3 K photon flux. This inconsistency still remains a problem as the arrival direction analysis does seem to suggest an extragalactic origin for these very high energy cosmic rays.

In general then cosmic ray research still appears to be a hive of activity with plenty of promise. However, progress is inevitably slow, perhaps reflecting the difficulties of both technique and interpretation. □

Efficient bumblebees are almost specialists

from John Krebs

THE familiar sight of bumblebees gathering pollen and nectar from wild flowers is the basis of a recent study by B. Heinrich (*Ecol. Monogr.*, **46**, 105; 1976), in which he analysed the foraging behaviour of individually marked worker bees of the genus *Bombus*. The bee uses different techniques to collect pollen and nectar from different plant species, for example it collects pollen from dogwood by pressing its body on the flat inflorescence, while on the cranberry it hangs upside down and shakes the pollen onto its body. The bees in Heinrich's study area preferred certain flower species over others: *Chelone* being the most favoured and *Solidago* the least. In fact the rank order of preference (measured by the number of bees per flower) corresponded exactly to the rank order of potential nectar yield from the various species—I will return to this point later. While this picture applies to the whole bee population, each individual bee specialises on a particular plant species (its 'major') and occasionally visits one or two other species ('minors'): the overall distribution of foraging effort by the bees is the sum of many specialists on different plants.

Specialisation is not purely a result of attachment to one particular site, nor is it temporary; many workers probably retain the same 'major' throughout their life of four to six weeks. The 'minors' of individual bees are also constant, but Heinrich found that he could induce workers to adjust their preference by adding sugar droplets to a 'minor' in their repertoire, which then became the 'major', showing that the choice of a 'major' is related to how profitable different plants are. The benefit to a bee of specialising was revealed in an experiment in which Heinrich compared the efficiency of experienced and naive bees in extracting nectar from monkshood, which is unusual in having its nectar hidden in two modified petals. The naive bees, which had foraged on other flowers but not monkshood, had difficulty in finding and extracting the nectar while the specialists did not. This and other observations suggested that a non-specialist bee would be rather unsuccessful in competing for nectar and pollen with a specialist.

Heinrich's results pose two intriguing questions: Why do all bees not major on the most profitable flower species? And why do individuals maintain minors in their repertoire? The answer to the first question is straightforward. As I have mentioned, the bee population as a whole distributed its foraging effort according to the order of profitability of the various flowers, so that the commonest major is the top ranking nectar source, the next commonest major the second ranking plant and so on. It also turns out that after the foragers have exploited an area, all the different plants contain about the same amount of left-over nectar—they are all reduced to the same level of profitability. This means that the lower nectar content of poor quality flowers is (more or less) exactly compensated for by the fact that more bees compete for nectar in the rich flowers. (This pattern of harvesting, which has also been observed in birds, is predicted by theoretical models describing how a maximally efficient forager ought to behave, but more of that another time.)

The question of why bees maintain minors is more difficult, but G. Oster and B. Heinrich (*Ecol. Monogr.*, **46**, 129; 1976) have developed a model to offer a tentative explanation. Their argument goes like this: if there is an array of flowers giving different quantities of nectar per unit effort, an efficient worker with perfect knowledge should forage purely in the most profitable flowers. When the bee has no previous knowledge, it has to sample the flowers to estimate their profitabilities and then adopt the "pure strategy" of foraging in the best

flowers. But suppose Nature is awkward, and the different flower types continually change ranks, what should the bee do? In the extreme case, when Nature obeys Murphy's Law and what the bee thinks is best immediately becomes the worst, a "mixed strategy" is best because it insures against a sudden change. Assuming that Nature is perverse but not too perverse, the bee's best strategy lies somewhere between mixed and pure: majoring with one or two minors. □

Photobiology

from R. P. F. Gregory

The Seventh International Congress on Photobiology was held in Rome on August 25–September 3, 1976.

WHAT advances stand out after four years' study of photobiology? G. Porter (Royal Institution, London) gave one answer in terms of picosecond kinetics. The primary processes of light-absorption by a pigment and its re-emission in fluorescence can now be studied at times approaching a few picoseconds using the streak camera. (The absolute theoretical barrier was set by the Uncertainty Principle in the region of 0.5 ps.) Both by advances in the understanding of the behaviour of excited states of pigments and the application of ultrashort pulsed laser spectroscopy, models have been produced of the operation at the nanosecond timescale of three photobiological pigments. M. Ottolenghi (Hebrew University, Jerusalem) described the interconversions of rhodopsin, bathorhodopsin and isorhodopsin in terms of the energy level diagram and was able to account for the observed quantum yield. Remarkably similar behaviour was reported for the analogous pigment bacteriorhodopsin from the typical phototroph *Halobacterium* (R. H. Lozier and colleagues, Ames Research Center, California). The phototransformations of phytochrome, preceding the appearance of the Pr and Pfr forms, are also in the model stage (R. E. Kendrick, University of Newcastle-upon-Tyne). Outside the formal sessions it was apparent that the problems of pigment organisation and the spectroscopic methods presently available for their investigation were common to these topics and also to the study of chlorophyll, both in membranes and in pigment-protein complexes. At this level Photobiology very clearly exists as a discipline.

The importance of flavins or flavo-proteins in many responses of organisms to light is becoming apparent, and the much debated alternative, carotene, is on the retreat, in such examples as the flagellar response of *Euglena*, and the development of the coleoptile of oat seedlings. Flavoproteins also appear to provide an explanation for the various 'blue-light effects' in mitochondria, *Neurospora* and the alga *Protosiphon botryoides*. In several cases there is clear evidence that the flavin is coupled to an electron transport system, such as a *b*-cytochrome or plastocyanin. In these examples the metabolic links between the action of the pigments and the observed effects on the organisms were relatively obscure; however, in photosynthesis there have been impressive developments in metabolism. These cover both the chloroplast-cytoplasm relationship of the normal (C3) system and the agriculturally-wasteful process of photorespiration. H. R. Bolhar-Nordenkampf (The University, Vienna) described techniques of measuring photosynthesis and photorespiration in crop leaves, from which the behaviour and future yield of the crop could be assessed. The possibility of photorespiration being an essential detoxification system for the various oxide radicals continues to be discussed.

The subject very clearly divides between the above 'natural, positive' aspects, and the adventitious and negative effects of light, particularly ultraviolet. Although there is considerable data on the effects of ultraviolet on proteins and nucleic acids, the carcinogenic action on human skin could not be simply explained. A more satisfactory molecular explanation could be claimed concerning the success in treating certain skin diseases such as psoriasis and mycosis fungoides with psoralens (which bind to DNA) and UV-A light (M. A. Pathak, Harvard Medical School). Not only ultraviolet, but visible light was clearly shown by J. Marshall (Institute of Ophthalmology, University of London) to lead to striking degradation of retinal cones in animals and people subjected to artificially extended daylength.

The contribution of photobiology to economic problems occupied much attention, in that in principle solar energy remains the one large source of energy available for immediate use. Biological conversion was discussed by D. O. Hall (King's College, London) who pointed out that in many areas existing knowledge and technology could provide not only increased food crops but materials such as ethanol and glycerol at competitive prices. In several cases it had been shown that producing crops under cover in large

areas with varied CO₂ levels not only relieves losses by photorespiration, but also increases nitrogen fixation and water economy. W. J. Oswald (University of California, Berkeley) is of course unchallengeable in his presentation of algal sewage-farming at a profit. A. A. Krasnovsky (Academy of Sciences, Moscow) and others set out possible photoconversion systems based on isolated pigments or stabilised membrane systems, provided with enzyme systems, for the production of hydrogen. It remained doubtful, however, whether simulated photosynthesis for the production of, essentially, heat would be competitive with existing 'solar panels' for heating (and refrigerating). The discussion of this topic (as Porter had the opportunity to point out in the Farrington-Daniel memorial lecture) has had a phenomenal growth rate in the past four years, and raised Photobiology to the status of a political issue. □

Sicily as the sum of parts

from Peter J. Smith

THE notion that Sicily straddles lithospheric plates is by now a familiar one. The crystalline massif forming the extreme northeast of the island is a continuation of the Calabrian metamorphic belt and thus linked geologically with Europe. The Ragusa carbonate platform in the extreme southeast, on the other hand, is an extension of the Sahara platform and thus linked geologically with Africa. The boundary between the African and European plates (see McKenzie, *Geophys. J.*, **30**, 109; 1972) probably runs along the northern edge of Sicily, except in the east where it lies south of the massif.

This interpretation places the re-sedimentation basin and nappes which form the western and central parts of the island firmly on the African plate. Indeed, Barberi *et al.* (*Earth planet. Sci. Lett.*, **22**, 123; 1974) were apparently able to confirm this when they showed palaeomagnetically that, the crystalline northeast apart, Sicily has been part of the African plate since the Cretaceous. But as might be expected from Sicily's marginal position, things may not be that simple. Caire (in *Gravity and Tectonics*, Wiley, 1973), for example, has suggested that there may have been a second intra-island plate boundary isolating the Ragusa platform—a possibility apparently not excluded by

the work of Barberi and his colleagues who analysed rocks only from the extreme east.

To test this idea, Schult (*Earth planet. Sci. Lett.*, **31**, 454; 1976) has now carried out a palaeomagnetic study of Upper Cretaceous and Middle Jurassic volcanic rocks from the west of Sicily. The two poles thus obtained are not only significantly different from those of comparable ages for Europe (as expected) but are even further removed from those for Africa and the Ragusa platform. Since the Upper Cretaceous, western-central Sicily has apparently rotated clockwise by about 90° with respect to southeastern Sicily (and hence with respect to Africa) and by about 60° with respect to stable Europe.

Schult admits that the margin of error on his results is larger than one would wish; but taken at face value the new poles confirm Caire's proposal that western-central Sicily and the Ragusa platform once behaved as, or as part of, separate crustal blocks. And implicit in that confirmation is western-central Sicily's unique sense of rotation. All other known crustal block rotations in the central Mediterranean were apparently anticlockwise. □



A hundred years ago

ARE WE DRYING UP?

SUCH is the title of a paper in the September number of the *American Naturalist*, by Prof. J. D. Whitney, the object of which is to bring together some of the more striking facts in regard to the desiccation of the earth's surface—or at least of a considerable portion of it—which has taken place in the most recent geological period, and to suggest the inquiry whether we have any proof that this desiccation has been and is continued into the historical period: in short, Are we drying up?

There is a prevailing popular impression that the countries around the Mediterranean are drier than they were two or three thousand years ago, and that this change is due in part, if not wholly, to the cutting down of the forests which are assumed to have once existed there. Yet, when this matter comes to be investigated, it would appear that there is little if any evidence either that there has been any such wholesale stripping of wooded lands, or that there has been any considerable change in the climate of that region.

From *Nature*, **14**, October 12, 527; 1876.

articles

Stratospheric aerosols and climatic change

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Global heat balance calculations imply that stratospheric aerosols, generated by volcanic explosions, have made important contributions to some observed climatic changes but that such aerosols generated by supersonic transports and Space Shuttles are unlikely in the next few decades to have a significant climatic impact.

THE stratosphere contains a layer of submicron-sized particles, centred at an altitude of ~ 20 km and extending from the tropopause to an altitude of ~ 30 km (ref. 1). Usually, the particles of this layer are made primarily of a concentrated sulphuric acid solution^{2,3}, which is generated *in situ* from sulphur-containing gases that undergo a series of oxidation and hydration reactions¹. At present, volcanic explosions are very important sources of these aerosols because of the sulphurous gases they inject into the stratosphere⁴. Volcanic explosions also introduce silicate particles into the stratosphere^{4,5}. Because the gas-particle conversion has a time constant ~ 1 yr (ref. 1), silicates are the dominant aerosol species in the first few months following a volcanic explosion⁵, but H_2SO_4 dominates for the next few years⁴. A future source of stratospheric aerosols may be from flight through the stratosphere. The effluent from supersonic transports (SSTs) includes sulphur-containing gases, which will be converted to H_2SO_4 , while the exhaust from Space Shuttles contain tiny aluminium oxide particles^{1,6,7}.

It is important to understand the climatic changes that might be produced by stratospheric aerosols. One variable useful in characterising the climate is the globally-averaged surface temperature, T , which depends on the global heat balance between the amount of solar energy absorbed by the entire Earth and the amount of thermal radiation emitted to space. Stratospheric aerosols scatter and absorb solar radiation and interact with and produce thermal radiation. Changes in their number will thus cause a temporary imbalance between the solar energy absorbed and the thermal radiation emitted to space and necessitate an adjustment in T to restore the global heat balance. We perform here accurate radiative transfer calculations to estimate the sensitivity of the climate to changes in the number of stratospheric aerosols. The results, when combined with observed changes in the level of volcanic activity, permit us to assess volcanism as a cause for past climatic changes. Calibrating by these findings, we calculate the climatic impact of future vehicular traffic through the stratosphere.

The model

We here summarise our radiative transfer calculations of T , specify the sources of our model's parameters, discuss factors not included in our calculations, and, where possible, introduce approximate corrections for these factors. It is not possible to evaluate the full climatic impact of changes in the number of stratospheric aerosols; but because the direct effect of these particles is radiative, our calculations provide a useful measure of the sensitivity of T to such changes.

For a specified model atmosphere, including a vertical profile of the aerosols, we determine the value of T that leads to global heat balance. Climatic change is measured by ΔT , the difference in T between the perturbed and unperturbed state. An accurate method, based on the doubling principle^{8,9}, was used to solve the multiple scattering problem for unpolarised light and so determine the amount of solar energy absorbed by the Earth. The thermal radiation emitted to space was found by numerical evaluation of the formal solution of the equation of radiative transfer¹⁰. For the solar calculations, account was taken of the absorption and scattering from both gases and aerosols. Because the single scattering albedo of stratospheric aerosols is small in the thermal infrared¹¹, scattering was neglected in the thermal calculations, but the opacity of both gases and aerosols was included. While no allowance was made for diurnal, latitudinal or seasonal variations in the model parameters, we did include vertical inhomogeneities. Also, tropospheric water clouds are present over 50% of the globe. A more detailed discussion is given elsewhere^{4,7,8}.

An increase in the amount of stratospheric aerosol can lead to either a warming or cooling of the Earth. Our radiative transfer calculations, as well as those of others¹², indicate that the sign and magnitude of ΔT depends critically on the real and imaginary parts of the refractive indices of the added particles, their size distribution, and the surface albedo. We use the measured refractive indices for materials constituting the aerosols of interest¹³⁻¹⁵ and size distributions based upon available measurements¹¹. Finally, we use a value of 0.1 for the surface albedo¹⁶.

The increase of the optical depth of the stratosphere, $\Delta\tau$, caused by the addition of particles has a strong effect on the magnitude but not the sign of ΔT . This parameter is proportional to the number of added particles which are large enough to interact efficiently with light. Figure 1 illustrates the variations of $\Delta\tau$ over the past century, as determined from observations of the amount of unscattered sunlight and starlight transmitted by the atmosphere⁴, and many of the observed

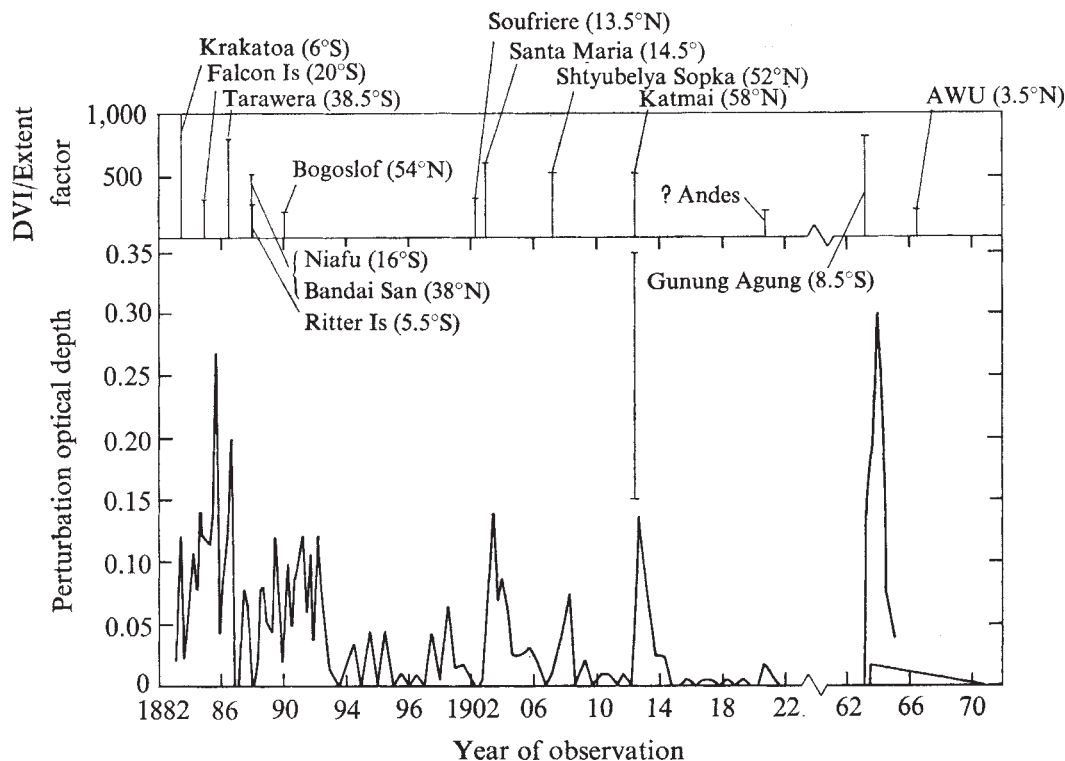


Fig. 1 Optical depth perturbations of the atmosphere at a wavelength of $0.55 \mu\text{m}$ as a function of time, and volcanic explosions that Lamb²⁵ has judged to have injected significant quantities of dust into the stratosphere. The dust veil index (DVI) is Lamb's assessment of the importance of an eruption. The larger perturbation in 1963 is a Southern Hemisphere value, and the smaller a Northern Hemisphere value. The error bar in 1912 represents a measurement at one location of a single day's perturbation and a one-month average. The solid curve is an average of monthly mean optical depths at several locations. Note the phase lag between the times of eruption and peak optical depths—partly because of the slow transport of dust from the point of injection into the stratosphere and partly because of the slow photochemical conversion of sulphur-containing gases to sulphuric acid aerosols, as is observed directly³⁷.

changes in $\Delta\tau$ can be associated with major volcanic events. We estimated $\Delta\tau$ for other times from less direct information, as indicated in the footnotes to Tables 1 and 2. In making these latter estimates, we have neglected any variation in the stratospheric residence times of the aerosols, which could result from changes in the stratospheric temperatures.

Our calculations neglect several feedback mechanisms that might augment the temperature changes (alterations of the snow and ice cover), retard these changes (an alteration in the dynamical heat transport), or has either effect (variation in cloudiness). Current climate models that include the first two of these feedback mechanisms suggest they cause an amplification by a factor of 4 in ΔT for transitions from interglacial to glacial conditions^{17,18}. Since 10^3 – 10^4 yr are required to establish the great continental ice sheets^{19,20}, we multiply our results by this factor when dealing with changes on time scales $> 10^3$ yr, and use a smaller factor for time scales as short as a few hundred years²¹.

Our calculations are also limited to the steady state, in which perturbation persists for a much longer period, P , than the response time of the land-atmosphere-ocean system, t . When dealing with time scales of a few months or less, when $P < t$ for both the troposphere and ocean⁴, we hold the tropospheric and ground temperatures constant and consider only temperature changes in the stratosphere. For somewhat longer time scales (still < 4 yr) $P > t$ for the troposphere, but $< t$ for the mixed layer of the oceans⁴. In this case we multiply⁴ our calculated value of ΔT by $P/2t$.

Volcanic activity as an agent for climatic change

Using this model, we have calculated ΔT for past, observed changes in the level of explosive volcanism and have compared the results with contemporaneous observations of ΔT . Table 1

summarises the results. The first two rows refer to the effects of single volcanic explosions, while the remaining rows refer to the effects of multiple volcanic explosions. The rows are also ordered according to the duration of the climatic change; the top row refers to the shortest time interval.

Comparison between the observed and predicted values for the first two rows of Table 1 provides a test of the validity of our model calculations. The calculated increase in the stratospheric heating rate agrees well with the increase observed during the first few months after the Mt Agung eruption. Also, the calculated decrease in the average surface temperature for the 2-yr period following an eruption is in accord with the observed decrease to within their mutual uncertainties. The net effect of volcanic explosions is to cause a temporary cooling of the Earth.

Rows 3 and 4 of Table 1 permit an assessment of the contribution of volcanic activity to the ΔT observed over the past century. This comparison is shown in greater detail in Fig. 2, where we have also indicated the ΔT expected from the observed increase in the CO_2 content of the atmosphere, coming primarily from the burning of fossil fuels^{22–24}. We have set the reference point of the temperature scale at 1885. The increase in T from the end of the nineteenth century until 1940, shown by the theoretical volcanic dust curve, arises from the declining level of volcanic activity over this period. Thus, the temperature was depressed during the early part of this period when there was much volcanic activity, but no such depression occurred during the later, volcanically quiescent times.

Figure 2 and Table 1 suggest that changes in the level of volcanic activity have made a significant contribution to the increase in surface temperature from the end of the nineteenth century to 1940, and a less significant contribution to the decline in temperature from 1940 to the present. Because we did not include possibly important feedback effects, such as variations in cloudiness, we cannot make a precise estimate

Table 1 Comparison between observed temperature changes and those expected from volcanic aerosols

Comparison		Characteristic of variable being compared			Model parameters				Comments
Observed value	Predicted value	Variable	Place	Time period	Aerosol species	$\Delta\tau$	F_1^*	$F_2^\dagger$	
0.1 (ref. 34)	0.1 $\ddagger$	Atmospheric heating rate $\S$	Southern Hemisphere, lower stratosphere	First several months after the Mt Agung eruption in 1963	silicate dust	0.1	—	—	The main source of the heating is absorption of outward-directed thermal radiation by the added aerosols rather than their absorption of solar radiation ⁴ .
-0.3 (ref. 26)	-0.2	Mean surface temperature change, $\Delta T^\P$	Northern Hemisphere	Epochal average for a 2-yr period after an eruption	sulphuric acid	0 as against 0.1	0.25	1	The observed value was obtained by averaging the results of 13 separate events. This averaged value is statistically significant at the 1% level.
+0.6 (ref. 35)	+0.5	Mean surface temperature change, $\Delta T^\P$	Northern Hemisphere	1880-1890 as against 1935-1945	sulphuric acid	0.07 as against 0	1	1	
-0.3 (ref. 35)	-0.15	Mean surface temperature change, $\Delta T^\P$	Northern Hemisphere	1935-1945 as against 1965-1970	sulphuric acid	0 as against 0.02	1	1	The agreement between the predicted and observed ΔT from 1940 to 1970 is worse than is apparent from this Table. See Fig. 2.
$\sim +0.5$ (refs 25, 33) -0.3-+0.6		Mean surface temperature change, $\Delta T^\P$	Northern Hemisphere	1450-1915 (Little Ice Age) as against 1935-1945	sulphuric acid	0.03** as against 0	1	1-2	The full ice-snow albedo feedback will not be realised since there was insufficient time to build large continental ice sheet. However, observations suggest that some increase in snow cover may have occurred during the Little Ice Age ²¹ .
+4 (refs 29, 33) +0.7-+7		Mean surface temperature change, $\Delta T^\P$	Both hemispheres	12,000-25,000 BP as against now	sulphuric acid	0.02-0.2 $\ddagger\ddagger$ as against 0	1	4	12,000-25,000 BP was the coldest part of the last ice age.

*Correction factor for the finite response time of the atmosphere-ocean-land system. A response time of 4 yr was used to calculate F_1 for the second row. This value was derived from the amount of mass in the mixed layer of the ocean^{4,33}.

$\dagger$ Correction factor for feedback from a change in ice and snow cover and in the atmospheric dynamics^{17,18}. This correction factor is poorly known.

$\ddagger$ The tropospheric temperature was held constant because the response time of the troposphere is several months^{4,36}.

$\S$ In units of $K\ d^{-1}$.

||Found from Fig. 1.

$\P$ In K. ΔT as given in the first and second columns of the table is the difference in temperature between the second and first time period of the fifth column. For example, the observed value of +0.6 given in the third row means T increased by 0.6 K from 1880-90 to 1935-45.

**The $\Delta\tau$ value for the Little Ice Age was found from Lamb's²⁵ dust veil indices (DVI). The DVI for this period are based primarily on estimates of the amount of mass ejected by volcanic events. We assumed that DVI is proportional to $\Delta\tau$ and found the constant of proportionality from data on both quantities for the period 1880-1915 (ref. 4). The $\Delta\tau$ given in Table 1 for the Little Ice Age is thus only a rough estimate.

$\ddagger\ddagger$ The values of $\Delta\tau$ were derived from the sulphate concentration found in ice cores²⁸. In deriving these values we assumed a residence time of 1 yr for the H_2SO_4 particles in the atmosphere, values of $30\ cm\ yr^{-1}$ and $15\ cm\ yr^{-1}$ for snow accumulation rates in Greenland and Antarctica, respectively^{29,28}, and the present 'normal' size distribution function of the stratospheric aerosols. The lower value of $\Delta\tau$ in the Table is a value found from the sulphate concentration for the Antarctic cores, while the higher value pertains to the sulphate concentration of the Greenland cores.

of these contributions. CO_2 was less important than volcanic aerosols in causing the temperature increase from the end of the nineteenth century until 1940. The relative ranking of these two effects, however, may well reverse itself during the next several decades and the Earth could experience a net warming over this period if no other factors intervene (such as man-made dust¹⁶).

During the period from ~ 1450 to 1915, the Earth was enveloped in the 'Little Ice Age'. Mountain glaciers and sea ice were more extensive than now, Northern Hemisphere temperatures were $\sim 0.5\ K$ colder than those for 1935-1945, and volcanic explosions were common²⁵⁻²⁷. The volcanic activity between 1880 and 1905 shown in Fig. 1 is typical of the Little Ice Age in terms of number and magnitude of eruptions²⁵. The results of Table 1 suggest that the high level of volcanic activity during the Little Ice Age had an important role in causing the low temperatures of this period.

Ice cores from Greenland and Antarctica contain information on both aerosol content and temperature^{28,29} that allow us to assess the importance of volcanic activity in generating and sustaining the last major ice age, the Wisconsin, which occurred

from $\sim 75,000$ BP to 12,000 BP. Because the residence time of submicron-sized aerosols in the stratosphere is ~ 1 yr, aerosols produced by a volcanic explosion are distributed uniformly over at least the hemisphere of the explosion, except for a short period immediately following the explosion^{4,12}. Thus, aerosol-derived material found in ice cores provides a useful measure of the aerosol content of the stratosphere at the time the ice was laid down. Studies of the anion content of ice cores²⁸ show that their sulphate content was distinctly higher than its present value and reached a maximum value during the 13,000-yr period at the end of the Wisconsin, a time when T had its minimum value. The sulphate enhancement was significantly greater for the Greenland cores than for the Antarctic cores. Sulphate also occurs in tropospheric aerosols, so an increase could result from non-volcanic sources. Assuming that the enhancement in sulphate content is caused by volcanic aerosols, we obtain estimates of ΔT for the later part of the Wisconsin ice age (Table 1) and find that volcanic aerosols could have made a significant contribution to the observed low temperatures.

Table 2 Estimated effect of vehicles flying through the stratosphere

Aerosol source	Time frame	$\Delta\tau^*,\dagger$	$\Delta T^\ddagger$
SSTs and other high-flying aircraft	1990 nominal	4.9×10^{-4}	5×10^{-3}
	1990 maximum	8.9×10^{-4}	9×10^{-3}
	2000 nominal	1.1×10^{-3}	1×10^{-2}
	2000 maximum	3.3×10^{-3}	3×10^{-2}
Space Shuttles	1990	3.3×10^{-5}	1.5×10^{-4}

*The Space Shuttle and SST perturbations are estimates for the Northern Hemisphere. Southern Hemisphere values should be 3/7 as large. SSTs are responsible for 70% of the $\Delta\tau$ values.

†The $\Delta\tau$ values were obtained from estimates of the number of vehicles, their emission characteristics, and properties of the stratosphere^{4,33}.

‡Temperatures (K).

Further support for this proposition comes from the discovery²⁹ of ~2,000 volcanic dust bands and ~25 volcanic ash bands in Antarctic ice cores. The small particle sizes ($\lesssim 5 \mu\text{m}$) in the dust bands suggest that the dust originated from distant sources. The observed onset of sustained volcanic activity occurred simultaneously with observed strong cooling, the lowest temperature accompanied the greatest dust fall-out, and a cessation of dust bands and an increase in temperature were contemporaneous²⁹. Finally, the average mass concentration of volcanic dust during the last part of the ice age implies the presence of a comparable amount of volcanic sulphate. This is consistent with the observed sulphate enhancement for the period.

While volcanic activity appears to have contributed to an intensification of the Wisconsin ice age during its later stages, the available evidence suggests that it had little to do with the early and middle portions of this ice age. Few dust bands occur in the Antarctic cores²⁹ for this period and little enhancement is seen in the sulphate content of the cores from both hemispheres²⁸.

At times earlier than the Wisconsin ice age, there are a few data that hint at a significant volcanic role in causing climatic change. A dramatic cooling known as the '90,000-yr BP' event has been detected both in polar cores and in sea cores. In the sea cores it was closely associated with a volcanic ash layer³⁰ and in the polar cores there was an order of magnitude more dust (not necessarily volcanic) during the event than at present³¹. Finally, studies of the number of volcanic ash layers in deep-sea

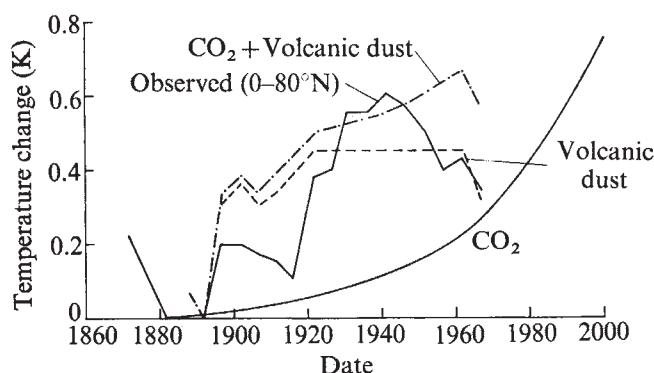


Fig. 2 Observed Northern Hemisphere surface temperature changes³⁵ (—) and theoretical estimates of the temperature changes from observed CO_2 and volcanic dust variations (---). CO_2 alone is the solid, steadily rising curve, and volcanic dust alone is ----. To estimate the volcanic temperature changes we use the optical depth data in Fig. 1 averaged over 10-yr periods and only consider the years 1884–1892, 1903–1904, 1907–1908, 1912–1914 and 1963–1970 to have any volcanic dust present. The 10-yr averaging period was chosen to allow for response times in the Earth's climate system. An even better fit, especially between 1900 and 1920, could be obtained by other choices of averaging period.

cores indicate that the worldwide level of volcanic activity during the Quaternary, the time of the ice ages, was much greater than during the previous 20×10^6 yr (ref. 32).

In summary, volcanic aerosols seem to have made important contributions to climatic changes in the past, but apparently do not offer a complete explanation of all recorded changes.

Possible climatic impact of stratospheric flight

In a manner analogous to our treatment of volcanically produced stratospheric aerosols, we calculated the ΔT from increases in stratospheric particles from SSTs and Space Shuttles operating at projected traffic levels for the next several decades^{6,7}. The results of these calculations are given in Table 2. Again, an increase in aerosol population in the stratosphere leads to a cooling of the Earth.

The importance of the ($\Delta\tau$, ΔT) values shown in Table 2 can be partially assessed by comparing them with $\Delta\tau$ s produced by volcanic eruptions and ΔT values associated with significant climatic changes. The largest $\Delta\tau$ that SSTs may produce is an order of magnitude smaller than the $\Delta\tau$ s produced by very minor volcanic activity, such as the Andes eruption of 1920 shown in Fig. 1. Thus, the contribution to the number of stratospheric aerosols by aircraft and rockets over the next few decades will be less than the contribution by random, quite insignificant volcanic eruptions.

According to Table 1, past climatic changes with $|\Delta T|$ values of 4 K and 0.5 K characterise the difference between glacial and interglacial periods and between the Little Ice Age and the decade 1935–1945, respectively. Clearly, a cooling of the Earth by 4 K would be disastrous for Mankind, since past ice ages have been characterised by huge sheets of ice covering almost all of Canada, most of the northern USA, and parts of northern and central Europe and the USSR. While it involved a much less dramatic change, the climate of the Little Ice Age was also significantly different from that of the present century. For example, the precipitation pattern in a number of marginal agricultural areas was notably different from the current one and the length of the growing season at high latitudes was significantly shorter³³. Thus, past climatic changes of as little as 0.5 K have had a serious practical impact. Changes of < 0.1 K would be less serious, however, than those that have occurred over the past several decades and would even be hard to measure. All the computed ΔT values of Table 2 are below this bound of 0.1 K; hence we conclude tentatively that the aerosols produced by vehicular traffic in the stratosphere over the next several decades will not have serious climatic consequences.

This conclusion can be put into some perspective by noting that about 70 Space Shuttle flights per day (against projections of < 1 per week) or 5,000 SSTs in operation (against projections of 250–1,500) would be required to decrease the surface temperature by 0.1 K. The conclusion thus reached on Space Shuttles is unlikely to be altered by future data, but the conclusion on the effect of high-flying aircraft should be re-evaluated as projections concerning their emission and flight characteristics are updated.

Finally, we note that the presence of additional particles in the stratosphere will have a negligible effect on the ozone cycle. For example, we estimate that the fractional increase in the ozone destruction rate due to scattering by the added particles is only ~0.1 of the $\Delta\tau$ values shown in Table 2 (ref. 7).

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The toxicity of ^{90}Sr , ^{226}Ra and ^{239}Pu

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Data now available on the risks of radiation-induced fatal cancer and hereditary disease and radionuclide metabolism suggest that limits on the rates of intake of ^{90}Sr , ^{226}Ra and ^{239}Pu at work, presently recommended by the International Commission on Radiological Protection, might be in need of considerable revision one with another and with the limit for uniform exposure of the whole body.

DISCUSSIONS on this subject often centre on the values of maximum permissible body burden (MPBB) recommended by the International Commission on Radiological Protection (ICRP)¹. For example, Spiers and Vaughan² considered the risk of osteosarcoma, cancer of the air sinuses and leukaemia arising from ^{226}Ra and ^{239}Pu in the skeleton, and they concluded that the currently recommended values of MPBB for these radionuclides do not need major revision. As Spiers and Vaughan² acknowledged, however, the recommendations of ICRP limit the rate of intake of radioactive materials into the body so that the MPBB for a well retained, long lived radionuclide will not be reached until the end of a working lifetime of 50 yr. In such circumstances, MPBB might be regarded more as an index by which to judge whether an individual has been overexposed, than as a quantity useful in planning for work with the radionuclide concerned. For this latter purpose, the corresponding maximum permissible annual intake (MPAI) is more pertinent. Values of MPAI may be obtained from the values of maximum permissible concentration in air or water (MPC) recommended by the Commission¹. It is worth noting that, even if there were no debate about the relationship between risk of an effect and dose, there might now be a need to revise values of MPAI because of the data on the uptake and retention of radionuclides in body tissues which have become available since the recommendations were made.

The recommended values of MPAI for each radionuclide are different for soluble and insoluble compounds and for inhalation and ingestion. Using methods similar to those described in the MRC report on the toxicity of plutonium³, we have estimated by way of example, the total risk of fatal cancer and serious hereditary disease arising from currently recommended values of MPAI for ingested soluble ^{90}Sr and ^{226}Ra and inhaled insoluble ^{239}Pu . We show that in these three cases the values of MPAI may be in need of considerable revision.

Relationships between risk and dose

Data on the risk of radiation-induced fatal cancer and hereditary disease have been reviewed by a United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR)⁴ and a National Academy of Sciences Advisory Committee on the Biological Effects of Ionizing Radiations (BEIR)⁵. Both reports adopt the hypothesis that the risk of an

effect is linearly related to dose. We have used the information in these reports to obtain the values discussed below and because they are based on a hypothesis which cannot yet be proved at the low doses concerned in radiological protection, they must be regarded as 'nominal' risks.

Leukaemia. Epidemiological studies on the survivors of the A-bomb explosions at Hiroshima and Nagasaki show an increasing leukaemia incidence with increasing dose. From the BEIR report⁵, the estimated incidence is 20–50 cases per 10^6 people exposed to 1 rad (20–50/ 10^6 /rad), in good agreement with the UNSCEAR estimate⁴ of 15–40 cases/ 10^6 /rad and the estimate of Smith and Doll⁶ of about 20/ 10^6 /rad. We have chosen a value of 30/ 10^6 /rad for radiation of low linear energy transfer (LET).

Breast cancer. Data on breast cancer incidence in women survivors of the A-bombs and in tuberculosis patients subjected to multiple X-ray fluoroscopies show an incidence rate of between 3 cases/ 10^6 /yr/rad (UNSCEAR) and 6 cases/ 10^6 /yr/rad (BEIR). We have assumed a 20-yr period of increased incidence to yield a morbidity of about 90 cases/ 10^6 /rad. Since the 5-yr survival of patients treated for breast cancer is about 50% (ref. 4) we have chosen a rounded value for the incidence of fatal breast cancer in females of 50/ 10^6 /rad of low LET radiation.

Lung cancer. The UNSCEAR report⁴ gives an estimate of 10–40 cases/ 10^6 /rad of high dose rate γ radiation from A-bombs during the first 25 yr after exposure. This is in good agreement with the value in the BEIR report of 0.9 cases/ 10^6 /yr/rem obtained from groups exposed to A-bomb radiations and to X rays in the treatment of ankylosing spondylitis. The value given in the BEIR report for exposure of the respiratory system to α rays from radon is 11.2 cases/ 10^6 /yr/rad for a follow-up period of about 25 yr. Using a value of 20 for the quality factor for α rays (*vide infra*) this corresponds to a total risk of about 14 cases/ 10^6 /rad of low LET radiation. Based on these two sets of data we have used a value of 20/ 10^6 /rad for low LET radiation.

Thyroid cancer. The UNSCEAR report estimates the incidence rate for thyroid cancer in adults to be in the range of 1 to 4 cases/ 10^6 /yr/rad and the BEIR report does not give a numerical estimate. We have assumed a value of 2.5 cases/ 10^6 /yr/rad and a 20-yr period of increased incidence, to give a total morbidity of 50 cases/ 10^6 /rad. The 5-yr survival from thyroid cancer, however, is about 80% (ref. 4) and we have chosen a value 10/ 10^6 /rad of low LET radiations for the risk of radiation-induced fatal thyroid cancer.

Osteosarcoma. There is little information on the induction of osteosarcoma by external radiation. From data on patients treated with X rays for ankylosing spondylitis, the BEIR report gives an estimate of 0.034–0.22 cases/ 10^6 /yr/rad average dose to bone over a follow-up period of 5–27 yr. The BEIR report also gives an estimate of 0.11 cases/ 10^6 /yr/rem average dose to bone for persons contaminated with ^{226}Ra . This value is based on a

quality factor of 10 for α particles and a follow-up period of 49 yr. It is, therefore, approximately equivalent to a risk of 110 cases/ $10^6/\alpha$ rad to cells on the endosteal surface of bone which receive about half the average dose in bone. This is in good agreement with the estimate by Spiers and Vaughan² of 95 cases/ $10^6/\alpha$ rad to the endosteum. We have, therefore, taken a risk estimate of 100/ 10^6 /rad to the endosteum for high LET radiation and 5/ 10^6 /rad for low LET radiation, the latter being consistent with data from the BEIR report and the quality factor of 20 for α particles (*vide infra*).

Air-sinus carcinoma. In persons contaminated with ^{226}Ra the risk of air-sinus carcinoma induction is about 40% of that of osteosarcoma², however, no such cancers have been induced in patients treated with ^{224}Ra (ref. 5). These data suggest that the air-sinus carcinomas arising in persons contaminated with ^{226}Ra are a result of irradiation of the air-sinus epithelium by ^{222}Rn and its short lived radioactive daughters trapped in the air cavities of the sinuses⁷. Therefore, we have assumed the risk of air-sinus cancer to be zero in persons exposed to ^{90}Sr and ^{239}Pu and to be 40% of the risk of osteosarcoma in persons exposed to ^{226}Ra (compare Table 3).

Other cancers. In the A-bomb survivors, an excess of cancers other than those listed here has been observed. This excess is not sufficient, however, to break down into specific categories. The estimate for these 'other' cancers by UNSCEAR was about 40 cases/ 10^6 /rad during the 25 yr after exposure and by BEIR about 2.0/ 10^6 /yr/rem. We have used a value 40/ 10^6 /rad of low LET radiation for the combined total of all 'other' fatal cancers. In the case of inhaled insoluble ^{239}Pu , the only 'other' tissue irradiated to any significant extent is the liver. For this organ we have used a risk estimate of 10/ 10^6 /rad of low LET radiation, derived from the MRC report³ when allowance is made for the different values of the quality factor used here and by MRC.

Serious hereditary disease. The risk of genetic effects from irradiation of the gonads is particularly difficult to estimate. Both the BEIR and UNSCEAR reports considered that the risk from irradiating oocytes is considerably less than that from irradiating spermatogonia. UNSCEAR derives a best value of 20 cases of serious hereditary disease per 10^6 in the immediate descendants of a male parent exposed to 1 rad of low LET radiation at low dose rate. Comparable estimates in the BEIR report for 1 rem to both parents, were 10–100 cases/ 10^6 in the first generation and 50–500 at equilibrium. BEIR also estimated the increase in congenital anomalies, anomalies expressed later and constitutional and degenerative diseases after both parents had received 1 rem, to be 1–100 cases/ 10^6 in the first generation and 10–1,000/ 10^6 at equilibrium. For workers exposed to radiation, the relevant risk is probably that of serious hereditary disease in their immediate descendants and for this we have chosen a value of 50 cases/ 10^6 for a worker receiving 1 rad of low LET radiation. The appropriate value for irradiation of the general population, if evaluated over many generations, would be greater, and greater still if diseases of less severity were taken into account.

Table 1 'Nominal' risk of fatal cancer and serious hereditary disease per unit dose

Effect	Tissue at risk	Cases/ 10^6 /rem	
		Male workers	Female workers
Leukaemia	Red bone marrow	30	30
Breast cancer	Female breast	—	50
Lung cancer	Lung	20	20
Thyroid cancer	Thyroid	10	10
Osteosarcoma	Cells on endosteal surfaces	5	5
Liver cancer	Liver	10	10
Other cancers	All other tissues	30	30
Hereditary disease	Gonads	50	50
Total	Whole body	155	205

The various risk estimates discussed above are summarised in Table 1 and are given per rem. In the calculations of dose equivalent discussed below it will be assumed that a quality factor (Q) of unity applies for low LET radiations and 20 for α particles as recommended⁸. The value of 20 for α particles is supported by cytogenetic work with ^{239}Pu (ref. 9) and experiments on chromosome damage in liver cells with low dose rate radiation¹⁰. A value of 20 is also compatible with the data on osteosarcoma induction mentioned above. ICRP used a value of 10 in its 1959 recommendations¹, as did the MRC³.

Dose commitment per unit activity taken into the body

Since it is assumed that risk of an effect is linearly related to dose, risk in any organ or tissue from intake of radionuclide is proportional to the resulting average total dose received by that tissue (the dose commitment) before induction of the effect. The tissues considered to be at risk are shown in Table 1. Their masses have been taken from the data given by ICRP for Reference Man¹¹. Metabolic models for the three radionuclides under discussion have been proposed in the reports of two ICRP Task Groups^{12,13}.

For inhaled insoluble ^{239}Pu we have used the lung model proposed in ref. 12 to calculate the dose commitment in lung (comprising the trachea, bronchi, pulmonary region and associated lymph nodes) and the fractions of ^{239}Pu transferred to the gut and the blood. We have assumed the inhaled particles to have an activity median aerodynamic diameter (AMAD) of 1 μm , however, differences in particle size within the range likely to be encountered in practice will not markedly affect the results³. The dose to the walls of the gut is small compared with that in other tissues because of the limited range of the α particles and the position of sensitive cells³. Of ^{239}Pu entering the blood, a fraction (0.45), is assumed to reach both bone and liver and be retained with half lives of 100 and 40 yr respectively¹². From data of Richmond and Thomas¹⁴ we have estimated that of ^{239}Pu entering blood $10^{-3}\%$ is deposited per g of testes or ovaries and remains there indefinitely¹⁵, and from experimental work in our unit, the dose to the spermatogonial stem cells is taken to be 2.5 times the average dose in the testes¹⁵. Our experimental work on female mice so far has not suggested that the dose to maturing oocytes is very different from the average in the ovary.

Average doses to cells on endosteal surfaces (taken to lie within 10 μm of the bone surface) and to haematopoietic bone marrow have been calculated for ^{239}Pu and ^{226}Ra using the Monte Carlo techniques described by Thorne¹⁶. ^{239}Pu was assumed to be distributed uniformly in a thin layer over all endosteal surfaces and ^{226}Ra in the volume of mineral bone (details of these calculations will be published elsewhere). Doses to the endosteum and red bone marrow from ^{90}Sr have been calculated using the data of Whitwell and Spiers¹⁷.

For ingested soluble ^{90}Sr and ^{226}Ra , the fractional absorption from the gut has been taken as 0.3 and 0.2 respectively¹³. The value for ^{90}Sr is a compromise between the value 0.2 given in ref. 13 and the mean value 0.38 given in ref. 11. Values for the time integral of activity of ^{90}Sr and ^{226}Ra in trabecular and compact bone and in the soft tissue of the body have been derived using data in ref. 13. ^{90}Sr is assumed to be in equilibrium with its short lived daughter ^{90}Y in all organs and tissues of the body at all times after ingestion. All ^{222}Rn produced from ^{226}Ra in the soft tissues of the body is assumed to escape from the body before it decays. Of ^{222}Rn produced in bone, 30% is assumed to remain at its production site, where it gives up the α energy of all its daughters except that of ^{210}Po , which follows the long lived ^{210}Pb . The remaining 70% is assumed to escape from the body without imparting any energy to tissues¹. For both ingested soluble ^{90}Sr and ^{226}Ra , the dose to the gut is very much smaller than that to the other organs and tissues shown in Table 2 and it has been neglected.

Values are given in Table 2 for the dose commitments in various organs and tissues of the body from ingestion of 1 μCi

Table 2 Dose commitments in various organs and tissues of the body from ingestion of 1 μCi of soluble ^{226}Ra , ^{90}Sr and inhalation of 1 μCi of insoluble ^{239}Pu

Tissue at risk	Dose commitment (rem μCi^{-1})		
	Ingested ^{226}Ra	Ingested ^{90}Sr	Inhaled ^{239}Pu
Red bone marrow	2	0.7	300
Female breast	0.4*	0.005*	—
Lung	0.4	0.005	1200
Thyroid	0.4	0.005	—
Cells on endosteal surfaces	20	0.9	3700
Liver	0.4*	0.005*	760
All other tissues	0.4	0.005	—
Gonads: Male	0.4	0.005	38
Female	0.4	0.005	15

* ^{90}Sr and ^{226}Ra are assumed to be uniformly distributed in soft tissues^{11,13}.

of soluble ^{226}Ra and ^{90}Sr and from inhalation of 1 μCi of insoluble ^{239}Pu . For the gonads, dose has been integrated over an assumed reproductive life of 20 yr and for other tissues, over a working life of 50 yr. These dose commitments therefore only apply to intakes which occur early in the working life.

Risk per unit activity taken into the body

Values of the 'nominal' risk per unit intake of the radionuclides were derived from the data in Tables 1 and 2 and are shown in Table 3. The risk estimates apply to intakes early in life and are overestimates of the true risk, in the sense that some cancers induced late in life will not be recognised because of their latent periods. Several aspects of the risks shown in Table 3 are worth noting.

(1) For ^{226}Ra a risk of leukaemia, about half that of osteosarcoma, seems at variance with human experience: in people containing the radionuclide, the incidence of leukaemia has been about ten times lower than that of osteosarcoma². A plausible explanation for this disparity might be that the use of an arbitrarily defined quality factor of 20 for α particles⁸ overestimates the risk of leukaemia relative to that of osteosarcoma. An alternative explanation concerns the distribution of cells sensitive to leukaemia induction within red bone marrow and the relationship between risk of an effect and dose for those cells. Cells in marrow at distances greater than the range of α particles (about 40 μm) from bone receive no dose from α particles arising in bone and cells nearer to bone receive increasing doses the nearer they are. Our estimates of the risk of leukaemia have been obtained from the estimated average dose in marrow and the observed risk of leukaemia per unit dose to the marrow of people who were uniformly irradiated either with A-bomb radiations or X rays. Therefore, our estimates will be in error if the sensitive cells are not uniformly

distributed in the marrow or if the dose-response relationship is not linear over the range of doses concerned. With regard to the latter, the lifetime doses to marrow which are of interest in radiological protection are within the range from which risk estimates were obtained^{4,5}. It is possible, however, that the assumption of linearity overstates the risk at low dose rates. Conversely, if a significant proportion of sensitive cells lie near to bone, we might have underestimated the risk of leukaemia because such cells receive doses much greater than the average in the marrow. We have experimental evidence that the incidence of myeloid leukaemia in mice reaches a maximum value at average doses in marrow of about 200–300 rad of X rays (I. Major and R. H. Mole, personal communication) presumably because at greater doses, the sterilisation of transformed cells exceeds their production. Smith and Doll⁶ have also drawn attention to this breakdown of the linear dose-response hypothesis to explain the absence of leukaemia in women treated for cancer of the cervix who received very large doses to small volumes of marrow¹⁸. In the radium cases exhibiting osteosarcoma², marrow cells near to bone received very large doses. If a significant proportion of marrow cells sensitive to leukaemia induction are near to bone, this might explain the low incidence of leukaemia observed. By contrast, it is interesting that osteosarcoma incidence in these radium cases was increasing at α -particle doses to cells within 10 μm of endosteal surfaces of 500 rad (10,000 rem) or more¹⁹. Clearly, more information is required about the distribution of the cells which are sensitive to induction of both osteosarcoma and leukaemia.

(2) For ^{226}Ra the predicted risk of hereditary disease is about 8% of the total risk, corresponding values for ^{90}Sr and ^{239}Pu are 1 and 2% respectively. These percentages would increase for populations exposed to the radionuclides for long periods.

(3) The risk of leukaemia predominates for ^{90}Sr . The relative risk of leukaemia to osteosarcoma for both ^{90}Sr and ^{239}Pu is much greater than would be expected from experiments on animals. Most of these experiments have, however, been conducted in ways which might not have favoured the incidence of myeloid leukaemia as discussed in (1) above.

(4) Although all three radionuclides are commonly referred to as "bone-seekers", these predictions show that risk arises mainly from irradiation of the skeleton and its marrow only in the case of ingested ^{90}Sr (98% of the total); for ingested ^{226}Ra , the risk from irradiating the skeleton is about 65% and for inhaled ^{239}Pu , about 45% of the total. In the last case, about 40% of the risk arises from irradiation of the lung.

We acknowledge that the estimates of 'nominal' risk we have made are open to criticism and that the data on which they are based have been interpreted in different ways (see, for example, ref. 20). There is also considerable uncertainty concerning estimates of dose and the relevant tissues at risk. Nevertheless, the estimates of 'nominal' risk (Table 3) were derived from the most recent data in official reports and it is interesting to

Table 3 'Nominal' risk per unit activity of soluble ^{226}Ra and ^{90}Sr ingested and insoluble ^{239}Pu inhaled, for male and female workers

Effect	Cases/10 ⁶ / μCi					
	Ingested ^{226}Ra		Ingested ^{90}Sr		Inhaled ^{239}Pu	
	MW	FW	MW	FW	MW	FW
Leukaemia	60	60	21.00	21.00	9,000	9,000
Breast cancer	—	20	—	0.25	—	—
Lung cancer	8	8	0.10	0.10	24,000	24,000
Thyroid cancer	4	4	0.05	0.05	—	—
Osteosarcoma	140*	140*	4.5	4.5	18,500	18,500
Liver	4	4	0.05	0.05	7,600	7,600
Other cancers	12	12	0.15	0.15	—	—
Hereditary disease	20	20	0.25	0.25	1,900	750
Total	248	268	26.1	26.35	61,000	59,850

* Increased from 100 to 140 to allow for the risk of air-sinus carcinoma in persons exposed to ^{226}Ra .
MW, Male workers; FW, female workers.

Table 4 Annual limits on exposure recommended by ICRP; the resulting values of MABB after exposure for 50 yr and the risk from 1 yr of practice exposed to the limits

Exposure	Annual limit	MABB (μCi)	'Nominal' risk (cases per 10,000 per year)
Ingested soluble ^{226}Ra	0.08 μCi	0.005	0.2
Ingested soluble ^{90}Sr	0.8 μCi	0.37	0.2
Inhaled insoluble ^{239}Pu	0.07 μCi	0.16	40
Irradiation of whole body	5 rem		9

consider the consequences of applying these estimates to current recommendations of the ICRP.

Body burdens and risk from the MPAIs recommended by the ICRP

The values of MPAI recommended at present by the ICRP for the radionuclides under discussion are shown in Table 4. They were obtained directly from the values of MPC recommended by ICRP, assuming that a Reference Man¹¹ takes in 2,200 cm³ of water and inhales 2×10^7 cm³ of air each day. Table 4 shows the maximum achievable body burden (MABB) which such a man could attain at the end of 50 yr if he were exposed to the MPAI each year for that period. The values for ^{90}Sr and ^{226}Ra of 0.37 and 0.005 μCi respectively have been obtained using the data given in ref. 13. About 90 and 80% of the ^{90}Sr and ^{226}Ra respectively, would be in bone and the remainder in soft tissues. These values of MABB are about 5 and 20 times less than those of MPBB recommended at present¹, namely 2 and 0.1 μCi for ^{90}Sr and ^{226}Ra respectively, of which 99% was assumed to be in bone. Values of MPC, and therefore of MPAI, were calculated to result in values of MPBB after a 50-yr exposure and the ratio of the presently estimated values of MABB to the recommended values of MPBB demonstrates the effect of using the new metabolic data now available¹³. No value of MPBB was given by ICRP¹ for inhaled insoluble ^{239}Pu , its intake being limited by the dose in lung. The value of MABB shown in Table 4 (0.16 μCi) was derived using the data from ref. 12. About 40% of this MABB will be in bone, about 30% each in liver and lung and only a small percentage in other soft tissues, including the gonads. The value of MPBB commonly used for ^{239}Pu (0.04 μCi) was derived by assuming that 90% of the activity would be in bone and is four times smaller than the value of MABB calculated here.

The average 'nominal' risks for men and women arising from the MPAIs are also shown in Table 4 and, for comparison, that arising from the dose limit of 5 rem to the whole body²⁰ recommended at present, assuming a 'nominal' risk of 180 cases per 10⁶ per rem, which is a representative value for men and women (Table 1). Since it is reasonable to expect that the risk from exposure to each of the various limits should be the same, the three values of MPAI and the dose limit for whole-body exposure are clearly in need of revision one with another. If it were agreed that the risk from exposure of the whole body

to 5 rem each year is acceptable to workers, then the MPAI for inhaled insoluble ^{239}Pu would have to be revised down by a factor of about five and those of ^{90}Sr and ^{226}Ra both revised up by a factor of about 50. The ICRP is at present revising its recommendations and will no doubt ensure that the hazards from occupational exposure will not exceed those that are accepted in most other industrial or scientific occupations with a high standard of safety (para. 47 of ref. 21). In this respect, the MRC report³, quoting a paper by Pochin²², compared the risk from an MPAI with the average risk of fatalities in other kinds of work for which there seems to be a dividing line at 1 in 10,000 per year between the relatively safe and less safe industries. Comparisons of this kind need to take account of many social and economic factors, discussion of which is outside the scope of this paper. Whatever may be the outcome of discussions on these matters, however, it seems likely that many values of MPAI will need to be revised, not always in the same direction, because of new data on the fate of radioactive materials after they have entered the body.

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Darwin's finches and the evolution of sexual dimorphism in body size

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A simple model based on certain bioenergetic phenomena accounts for seasonal patterns of sexual dimorphism, size related changes in the timing of reproduction and geographic variation in body size of Darwin's finches.

SEXUAL dimorphism in size influences the biology of birds in many ways; for example, differences in beak size determine the size and kind of food that a bird can capture and ingest¹⁻³. Consequently, monogamous pairs that differ in size may increase the rate at which they feed their nestlings, since the adults do not compete for the same food items^{1,2}. Interspecific aggression⁴

or intraspecific aggressive conflicts between males, where increased size could influence the outcome, may account for the general avian condition that males are the larger sex². In addition, sexual differences in size may facilitate pair formation^{5,6}.

Many observations indicate that sexual dimorphism in body size may be subject to another equally broad explanation. First, among monomorphic species, sexual differences in behaviour result in each sex foraging on different things in different places⁷⁻¹⁰. Second, full adult size is generally achieved at fledging, and certainly before first reproduction, so increased male size in polygynous species may reduce the risks associated with delayed sexual maturation in males¹¹. Third, sexually dimorphic species in which the female is the larger sex require special explanations¹²⁻¹³. Finally, since beak and body size are correlated, dietary differences associated with beak size may be an inevitable consequence of the advantages associated with size alone.

Here I examine the proposition that sexual dimorphism in body size influences the timing of reproduction in fluctuating and seasonal environments. Evidence favouring the hypothesis is drawn from general studies on the energetic consequences of differences in body size, Lack's studies on morphological variation in Darwin's finches, and from my own studies of the reproductive biology of *Geospiza conirostris* and *G. fuliginosa*, two seed-eating finches that inhabit Española, the southernmost of the Galápagos Islands.

The influence of body size

Body size (weight) influences the rate at which birds use energy. Resting metabolism, the energetic costs of flight¹⁶, and existence metabolism¹⁷ bear an exponential relationship to body weight as do egg weight¹⁸, heart rate and conductance¹⁹. In general, the exponent of these relationships is less than 1.0. In contrast, functions describing the storage capacity or energy reserves of a bird, for example, digestive capacity, and lipid reserves²⁰, bear a nearly linear relationship to body weight. These different relationships affect the ability of a bird to respond to periods of deprivation and surfeit.

Consider two birds differing only in weight that are subjected to a physiological stress such as fasting or water deprivation after having been maintained on an *ad libitum* diet. To withstand this period of stress, they draw on stored reserves. The rate at which the larger (R_L) or the smaller (R_S) individual uses energy is dependent on body weight and can be expressed as follows

$$R_L = aW_L^b$$

$$R_S = aW_S^b$$

and

where W_L and W_S are the weights of the larger and smaller individuals. Since a and b are constants, R_L is always greater than R_S .

How long (T_L , T_S) each individual persists, depends on the size of its reserve (cW_L^d and cW_S^d) and the rate at which energy is used

$$T_L = cW_L^d/R_L = \frac{c}{a} W_L^{(d-b)}$$

and

$$T_S = cW_S^d/R_S = \frac{c}{a} W_S^{(d-b)}$$

where c and d are constants. As long as d is greater than b , T_L will be greater than T_S and the larger individual will persist longer than the smaller. It also follows, that when confronted with a surfeit of resources, the smaller individual can replenish its reserves faster than the larger ($R_L > R_S$), provided that size does not influence the efficiency of uptake in any disproportionate way.

This analysis of the consequences of differences in body size is inherent in explanations of geographical variation in size of Atlantic salmon (*Salmo salar*)²⁰, cricket frogs (*Acris crepitans*)²¹,

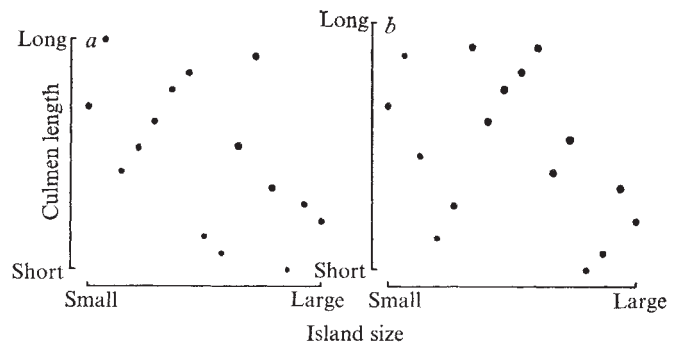


Fig 1 Rankings of culmen length and island size for populations of (a) *Geospiza fuliginosa* and (b) *Certhidea olivacea*. Culmen length data are taken from Lack²⁸; island sizes are given by Bowman²⁶. a, $R_s = -0.55$, $P < 0.05$, $n = 15$; b, $R_s = -0.44$, $P < 0.05$, $n = 16$.

wood rats (*Neotoma* sp.)²², and has been expanded in detail for birds by Calder¹⁹.

In fluctuating environments, consequences of differences in body size may become important. Among temperate species, periods of inclement weather alter foraging behaviour²³, and severe weather conditions can alter the survivorship of conspecifics that differ in body size^{24,25}.

Darwin's finches and the Galápagos

The lowlands of the Galápagos Islands are dry, hostile places, but on the larger islands these 'arid zone' forests give way to more mesic environments at higher elevations²⁶ and on these islands, Darwin's finches migrate from the arid coast to the highlands during periods of drought^{26,27}. On smaller, low lying islands, migration may be useless since the entire island may be covered with arid zone vegetation, so the finches are more likely to be subjected to periodic stress than would conspecific populations on larger islands. It follows from the analyses of the consequences of body size that finches on small islands should be larger than conspecifics on large islands.

Geospiza fuliginosa, the smallest of the seed-eating Geospizinae, occurs on many islands in the Galápagos, as does *Certhidea olivacea* the smallest insectivorous finch in the archipelago^{27,28}. In both cases, the birds on small arid islands tend to be larger than their conspecifics on larger islands (Fig. 1). Although this trend is consistent with the conclusion that increased size provides some resistance to environmental change, it is also consistent with the idea that size is determined by diet², since both seeds²⁹ and insects³⁰ are likely to be larger in more arid environments. The size and frequency distributions of seeds and insects on islands in the Galápagos are not known, but the effects of differences in prey size and climatic variability are complementary, in that both would select for larger size on smaller islands.

Body size and timing of reproduction

The reproductive patterns and life histories of vertebrates are also influenced by differences in body size. Large species generally mature slowly, produce small clutches, and are long lived, whereas smaller species mature more rapidly, produce larger clutches and are shorter lived³¹. The possibility that differences in body size would influence the timing of reproduction in seasonal environments has not been investigated.

Consider two species, with similar ecologies and competitive abilities, that differ in body size. On the assumptions that reproduction is initiated by a variable environmental cue and that the energy for egg production is derived from currently available food supplies rather than from stored reserves, it follows that females of the smaller species are likely to accumulate the necessary energy for egg production sooner than those of the larger species because they have a reduced maintenance cost¹⁹. The smaller species should, therefore, breed earlier than the larger species (Fig. 2a).

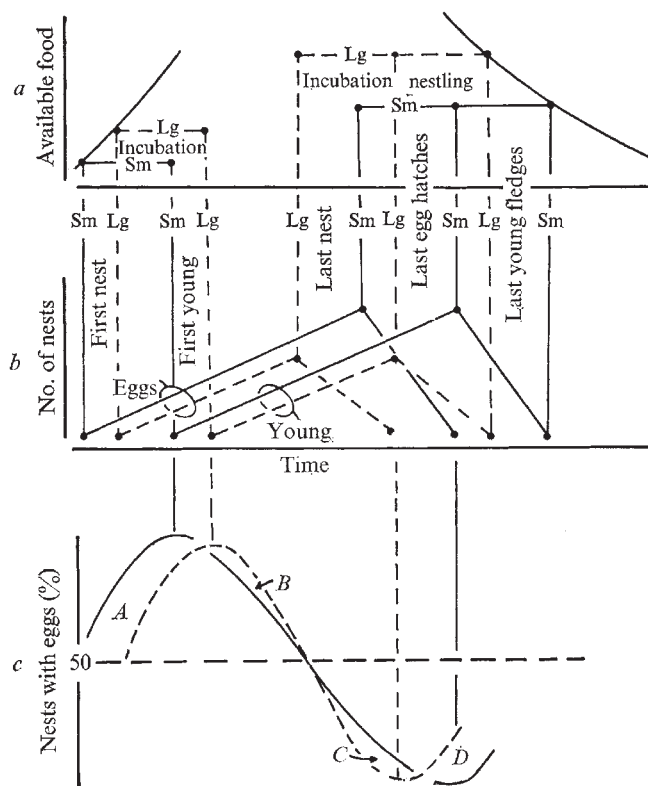


Fig 2 A graphic model for size-dependent changes in the timing of reproduction. Sm, Smaller species; Lg, larger species. See text for discussion.

If clutch size is similar for both species, then the larger species will require more food to support its young, since they are larger; and if the breeding season is terminated by a decline in available food, the breeding season of the larger species will end before that of the smaller. A major effect of size will thus be to shorten the breeding season for the larger species (Fig. 2a).

The differences in length of the breeding season for species of different sizes will alter the observed patterns of reproduction at the population level (Fig. 2b and c). Presume first that a few individuals are breeding throughout the year. When a breeding season starts, the establishment of new nests and production of new clutches will shift the relative proportions of nests with eggs away from 50% (Fig. 2c). The deviation of the population towards a surplus of nests with eggs will be countered as soon as the first eggs hatch. The same pattern will occur for the larger species, except that it will occur later. There is thus a period when the smaller species is in the incubation phase (more than half the nests have eggs) of a nesting cycle and the larger species is not (A in Fig. 2c). Since the larger species nested later, its population will seem to remain in incubation phase longer than the smaller species (B in Fig. 2c). As the end of the breeding season approaches, the larger species ceases establishing nests,

its breeding population is at a maximum level (Fig. 2b) and shifts into a nestling phase (nestlings present in more than half the nests) before the population of the smaller species does so (C in Fig. 2c). Since the smaller species can establish nests until somewhat later, it will not achieve maximum densities until then (Fig. 2b), and so it enters the nestling phase (D in Fig. 2c) later than the larger species.

My studies of the reproductive biology of two species of Darwin's finches, *Geospiza fuliginosa* and *G. conirostris*, provide a direct test of the foregoing model.

Darwin's finches on Española

I visited Española in July 1972 and again in January and February 1973. On both occasions I netted, weighed, measured and banded birds in the vicinity of Punta Suárez and searched that area for active nests (unpublished). Some birds were breeding during my first visit, but most breed during the rainy season (January–March)³². During my second visit, I collected crop samples from nestlings of both species. On Española, individual *Geospiza fuliginosa* weigh 14–16 g; whereas individual *G. conirostris* weighed from 25 to 39 g. Although these birds differed in body size, their breeding biologies were remarkably similar: both commonly incubated clutches of three eggs (range 1–5) and required about 30 d to complete a clutch and fledge their young; and both showed similar preferences for nest sites (my unpublished results) and the diets of their nestlings were also similar.

The diets of nestling *G. conirostris* contained 17% seeds with the remainder being composed of a variety of invertebrates. *G. fuliginosa* nestlings received a diet composed of 35% seeds. Both species fed the same kinds of seeds to their nestlings (Table 1). Of the over 5,000 seeds recovered from nestling *G. conirostris*, 95% by count and 88% by volume were of one species of grass, *Panicum hirticule*. This species also accounted for more than 86% of the >8,000 seeds recovered from *G. fuliginosa* young, and made up more than 80% of the volume of seeds in those samples. Thus, for both finches, the same grass species accounted for 80–90% of seed material that they fed to their nestlings.

Lepidoptera larvae of various sizes made up over 96% of the

Fig. 3 Frequency distribution for the sizes of lepidoptera larvae recovered from nestling *Geospiza fuliginosa* and *G. conirostris* in February 1973. Total no. of larvae: *G. fuliginosa*, 838; *G. conirostris*, 172.

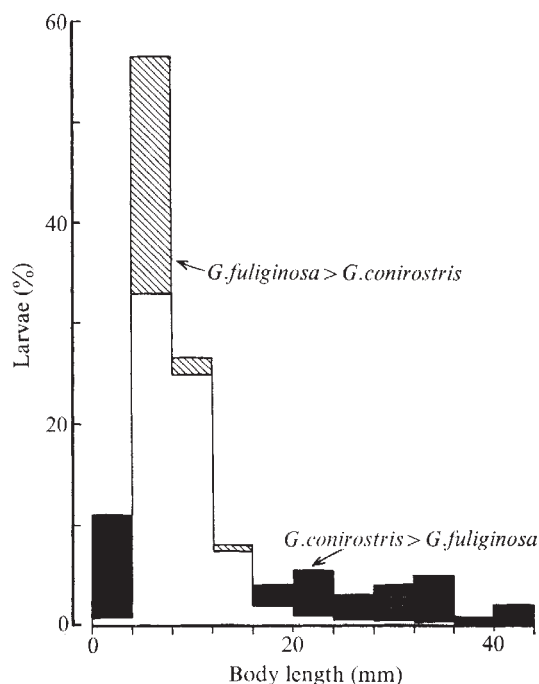


Table 1 The relative numbers of different kinds of seeds recovered from nestling *G. conirostris* and *G. fuliginosa*

Source	Seed volume (mm ³)	Percentage occurrence	
		<i>G. conirostris</i>	<i>G. fuliginosa</i>
Seeds smaller than <i>P. hirticule</i>	0.50 ± 0.029	0.6	
	0.13 ± 0.002		1.6
<i>Panicum hirticule</i>	0.69	93.6	86.5
Seeds larger than <i>P. hirticule</i>	1.10 ± 0.012	5.6	
	1.06 ± 0.005		11.7
Total number of seeds recovered		5,170	8,262

Table 2 Temporal changes in the diets of nestling *Geospiza conirostris* and *G. fuliginosa*

Date	Sample size		Nestling weights (g)		Animal matter in diet (vol %)		Samples (%) with large larvae (> 20 mm)		Samples (%) with small larvae (20 mm)	
	con.	ful.	con.	ful.	con.	ful.	con. only	con.	ful.	
Feb. 15, 16	5	35	21.2 (1.53)	12.9† (0.26)	95	73	80	20	82	
17, 18	26	19	22.2* (0.93)	12.5 (0.35)	91	75	50	42	85	
19, 20	18	15	22.1 (0.41)	13.3 (0.41)	72	62	56	61	81	
21, 22	7	21	22.1 (0.71)	13.4 (0.28)	76	61	14	43	95	
23, 24	10	20	24.3 (0.70)	12.8 (0.33)	85	58	30	80	85	
25, 26	8	13	24.4 (0.60)	12.6‡ (0.92)	71	63	25	68	92	
27, 28	—	8	—	13.7 (0.62)	—	62	—	—	88	
Mar. 1, 2	5	9	18.8 (2.17)	12.3 (0.94)	46	60	0	80	100	
Rank correlations with date			−0.22	−0.12	−0.86§	−0.60	−0.86§	0.90¶	0.73§	

* $n = 25$; † $n = 14$; ‡ $n = 7$; § $P < 0.05$; ¶ $P < 0.01$

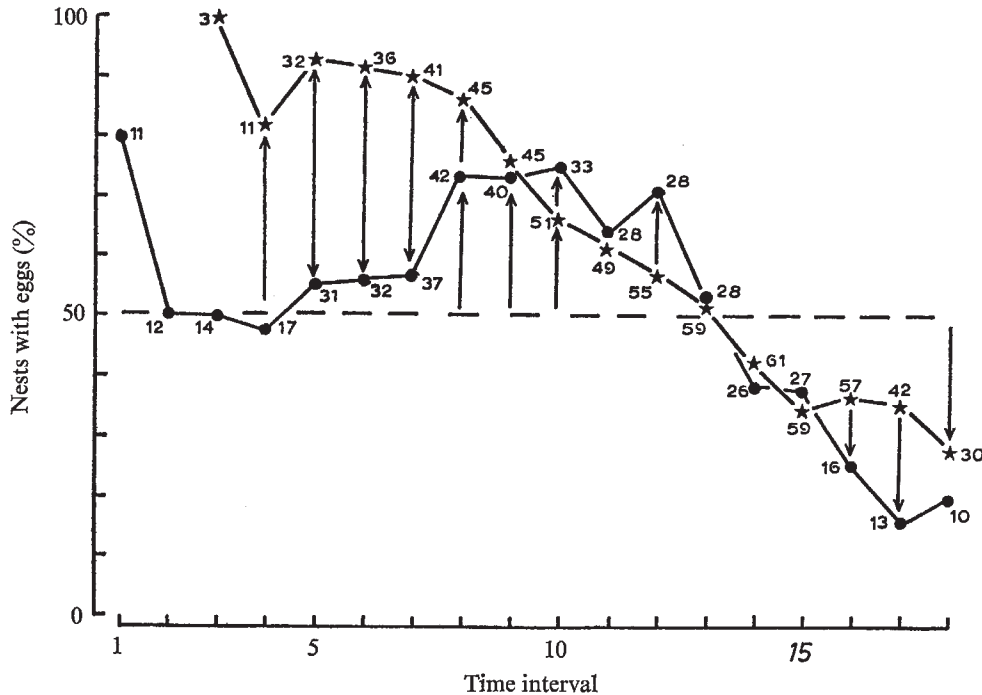
volume of invertebrates recovered from nestling *G. conirostris*, and 76% in the diets of *G. fuliginosa* young (Fig. 3). Small larvae were present in the diets of both species, but large larvae were more common in the diets of *G. conirostris* ($t = 4.04$, $P < 0.001$). Over 30% of the larvae recovered from *G. conirostris* nestlings were 20 mm long or longer, whereas larvae of this size accounted for less than 3% of the larvae in the diet of *G. fuliginosa* nestlings. In total, larvae of lepidoptera and the seeds of *P. hirticula* alone accounted for 96% and 80% (by volume) of the food items that *G. conirostris* and *G. fuliginosa* fed to their young.

Nestling diets changed during the course of my study (Table 2). Small larvae (< 20 mm in length) always composed 80% or more of the larvae recovered from nestling *G. fuliginosa* during the period February 15 to March 2; whereas, large larvae (> 20 mm) became an increasingly rare dietary item for nestling *G. conirostris* and small larvae became more common. This change influenced the overall dietary balance for nestling *G. conirostris*. In mid-February, diets of nestling *G. conirostris* contained 94% animal matter (by volume) whereas by March 2,

animal matter accounted for less than 50% of their diet. Thus, the diets of nestling *G. conirostris* showed a progressive deterioration late in the study, a change that was independent of nestling weight, and not observed in the diets of nestling *G. fuliginosa* (Table 2). These findings are not inconsistent with the assumptions and expectations of the model. The species are similar in a variety of ways, and changes in the nestling diets occur first in the larger species. The temporal patterns of reproduction for both species are also instructive.

It is likely that some breeding occurs throughout the year for both of these species (D. Werner and P. Kramer, personal communication). When I visited Española in January and began to follow the course of activities at various nests, neither species had as yet entered the incubation phase of the breeding cycle (Fig. 4). The *G. fuliginosa* population entered the incubation phase in interval 4 and that population was dominated by nests with eggs until interval 10. The incubation phase began later for *G. conirostris* (interval 8) and a significant majority of nests had eggs until interval 12. Thus, the incubation phase began earlier

Fig. 4 Temporal changes in the percentage of nests with eggs for *Geospiza fuliginosa* (★) and *G. conirostris* (●). An arrow indicates a significant departure from a 1:1 ratio. A double-headed arrow indicates that there are differences between the two species as well. The data are summarised at 2-d intervals beginning on January 22 and ending on March 2, 1973.



and terminated earlier for *G. fuliginosa*, the smaller species, than it did for *G. conirostris* (compare with Fig. 2c). Exactly the reverse pattern occurred for the onset of the nestling phase. The nestling phase for *G. conirostris* began in interval 16, whereas for *G. fuliginosa* it was delayed until interval 18 (again compare with Fig. 2c). Finally, as expected from the model (Fig. 2b), *G. conirostris* had completed a maximum number of nests (interval 8) before *G. fuliginosa* (interval 14). In short, the observed nesting pattern in both species is identical to that expected from the model and suggests that the larger species, *G. conirostris*, does, indeed have a shorter breeding season than its smaller counterpart.

Seasonal sexual dimorphism in *Geospiza conirostris*

The analysis initially considered comparisons between species because size-related differences in their biology are more easily detected, but the model applies with equal force to individuals of the same species but different size.

During a major breeding season, all females are likely to breed, and so it would be difficult to determine if smaller females of the same species were breeding earlier, later, or were more likely to re-nest. If a short breeding season occurs, however, then females of small size may breed when the large ones do not.

The Galápagos are affected by the *el niño* phenomenon³³. This event, which is tied to movements of ocean currents, has the effect of producing wet season (December–March) rains during the dry season (June–August) and thereby creating conditions similar to those in the breeding season. My first visit to Española occurred during an *el niño* year, therefore a comparison of the size of the birds breeding in July with those breeding in January provides a further test of the model.

In July 1972, I netted 225 *G. conirostris*; of which 17 were females with brood patches, and 14 were males in all-black plumage. During the same visit, I located five active *G. conirostris* nests and 15 active *G. fuliginosa* nests. During my winter visit, I netted 231 *G. conirostris*; of which 75 were females with brood patches, and 43 were mature males. In addition, I located 99 *G. conirostris* nests, and the male at 80 of these successfully attracted a female. The occurrence of few active nests in July suggests that *G. conirostris* had recently ceased breeding and the paucity of adults in reproductive condition indicates that only a few individuals attempted to breed in July.

During the January–March breeding season there was no difference in size between males and females (Table 3). Although the mean size of females was less than that of males, the difference is insignificant. In contrast, however, the females breeding in July were significantly smaller than males both in body weight ($F_{1,30} = 12.5$, $P < 0.001$) and in culmen (beak ridge) length ($F_{1,30} = 5.15$, $P < 0.05$). Further, the females in breeding condition in July were significantly smaller than females that bred in January, both in body weight ($F_{1,91} = 6.78$, $P < 0.01$) and in bill size ($F_{1,91} = 6.60$, $P < 0.05$). Again the observed pattern was as expected from the model.

The extent to which smaller females will have additional opportunities to breed will depend on the frequency with which differential cues occur. The *el niño* vagaries of the Galápagos climate ensure that these events occur with some regularity. A consequence of this phenomenon is to depress the mean size of

females in the population, thus producing a sexually dimorphic population with regard to size.

The seasonal pattern of sexual dimorphism documented for *G. conirostris* and its suggested causes are not limited to large species, rather they should affect all species of finches in the archipelago. This seems to be the case. Lack^{27,28} documented, but did not explain, the existence of sexual dimorphism in size in all except one of the Geospizinae—the Cocos Island finch (*Pinaroloxias inornata*), a species restricted to a humid tropical island just off the west coast of Costa Rica.

Size dimorphism in birds

Sexual dimorphism in size may result in differential resource partitioning^{1,2,34}. Such partitioning occurs in Darwin's finches³⁵, but if it were a pervasive selective pressure for sexual dimorphism, it should manifest itself in assortative matings during the breeding season. That is, the male in each pair should consistently be larger, or heavier, than his mate. I have measurements on 12 pairs that bred in January. The male had a larger bill in five instances and was heavier in seven instances. Thus, although the sample is small, it gives no indication that assortative mating was occurring.

Sexual dimorphism has also been attributed to selection for increased size in males, which may be advantageous in male–male competition for breeding sites. In contrast, the finch data indicate that decreased female size resulted in increased fecundity in a fluctuating environment. In particular, small size in females increased their sensitivity to environmental changes that indicate favourable conditions for breeding. Smaller females are thus likely to breed sooner and more often than their larger counterparts.

My inferences concerning sexual dimorphism and its effect on the timing of reproduction in Darwin's finches are based on the consequences of certain bio-energetic relationships and depend on when the resources used for egg production are accumulated. If they are accumulated immediately before reproduction, then relatively small females will be at an advantage because they can accumulate the necessary resources more quickly. For small passerine birds, this is likely to be the case, and in house sparrows (*Passer domesticus*) in particular, Johnston and Sealander³⁶ have documented a trend of increasing sexual dimorphism with latitude, a finding which is consistent with the model since the timing of reproduction should be more critical in places with short growing seasons. Conversely, if resources for reproduction are derived from reserves stored at some other time, say, before migration, then larger females will be at an advantage because they will have more reserves left for egg production. It is notable that the degree of 'reversed sexual dimorphism' in the three species of Accipiters studied by Storer¹³ increases with the degree of migration. Furthermore, certain species of shore birds, in which females are larger than males, arrive on their sub-arctic breeding grounds with substantial fat reserves³⁷, and the largest females breed first⁶. The timing of reproduction seems to be critical for these species; the arctic growing season is short and early breeding is associated with increased reproductive success³⁸. In addition, Brodie³⁹ has detailed an identical explanation for reversed sexual dimorphism in fin whales (*Balaenoptera physalus*), in which the females store fat while feeding on krill in the Antarctic. They subsequently migrate northwards to their breeding grounds, which are characterised by warmer temperatures and reduced food productivity, where females give birth to and nurse their calves. The energy used during gestation and lactation seems to be derived from fat reserves stored in the Antarctic, and the larger size of females endows them with increased storage capacity to satisfy the demands of reproduction.

Larger male size is characteristic of many species of birds. In certain North American species, males do not migrate as far South as females and overwinter in harsher climates, where increased size may reduce overwinter mortality. That migratory pattern seems to ensure the early arrival of males on the breeding ground⁴⁰. In polygynous song birds, males generally arrive well

Table 3 Seasonal sexual dimorphism in *Geospiza conirostris*

Character	Date	Males		Females	
		n	Mean	n	Mean
Body weight (g)	July 1972	14	32.9 (0.66)	17	29.6 (0.62)
	January 1973	43	32.3 (0.37)	75	31.7 (0.34)
Culmen length (mm)	July 1972	14	14.0 (0.21)	17	13.3 (0.24)
	January 1973	43	14.1 (0.12)	75	13.9 (0.10)

Standard errors are given in parentheses.

in advance of the females and establish territories⁴¹⁻⁴³. As a consequence, males are subjected to the rigours of territorial establishment at a time when the weather is likely to be inclement. Agonistic encounters are likely to reduce the time available for foraging and thus larger size may provide males with the physiological reserves necessary to hold a territory. This certainly seems to be the case in polygynous mammals. Male northern fur seals (*Callorhinus ursinus*) are 7-10 times the size of females⁴⁴, and establish territories before the arrival of the females. Territorial bulls fast for the duration of breeding season (2-4 months)⁴⁵; Bartholomew⁴⁶ interprets the extreme sexual dimorphism in this species to be a result of the advantages of early territorial establishment and prolonged territory maintenance, advantages that could be realised by the increased storage capacities associated with increased size.

In summary, simple bioenergetic considerations provide a general description of geographic patterns of size variation, size-related changes in the timing of reproduction and seasonal patterns of sexual dimorphism in Darwin's finches. Expansion of these analyses provides a consistent explanation for patterns of normal and reversed sexual dimorphism in birds and mammals. The analysis accounts for the general condition of larger male size in temperate species and identifies the conditions that would lead to increased female size. In all certainty, these initial tendencies will be amplified by other selective pressures².

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Chemical characterisation of the Thy-1 glycoproteins from the membranes of rat thymocytes and brain

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The Thy-1 antigens from both thymocytes and brain of rats are major membrane glycoproteins of about 25,000 molecular weight of which 30% is carbohydrate. The brain and thymus glycoproteins contain very similar amounts of each amino acid, but have strikingly different carbohydrate compositions. The antigenic determinants are likely to be in the protein part of the molecule.

MANY specific functions of cells are likely to be mediated by tissue specific molecules at the cell surface reacting with other cells or soluble factors. One method of identifying these is to study tissue-specific cell surface antigens, which are commonly referred to as differentiation antigens^{1,2}. The theta (θ) antigen, now called Thy-1 (ref. 3) was one of the first cell-surface differentiation antigens to be identified on lymphocytes, and was found in mice in two allelic forms which were serologically identified as the Thy-1.1 (θ -AKR) and Thy-1.2 (θ -C₃H) alloantigenic determinants⁴. The Thy-1 locus is on chromosome 9 in the mouse⁵.

The Thy-1 antigen was found in large amounts on mouse thymocytes and brain^{4,6} and in smaller amounts on mature T lymphocytes^{7,8}. The number of antigenic sites per thymocyte was found to be >400,000 for Thy-1.2 (ref. 8) and about 600,000 for Thy-1.1 (ref. 9) by measuring binding of the alloantibodies. The antigenic activity of brain is approximately equal to thymocytes on a packed tissue basis⁴. The Thy-1 antigen has also been reported on mouse epidermal cells and fibroblasts^{10,11}, but other tissues have very little if any antigen⁴. In the brain the Thy-1 antigen is present in small amounts at birth and increases to maximum specific activity after about 25 d (ref. 6).

The Thy-1.1 antigenic determinant can be recognised on rat thymocytes and brain with mouse alloantiserum, but no rat strain with the Thy-1.2 determinant has been reported¹². The amount of antigen on rat and mouse thymocytes and brain in similar as is the developmental pattern in the brain but, surprisingly, most peripheral rat T cells lack the antigen, while many rat bone marrow cells express it at the cell surface^{2,9,13}. Other tissues of the rat including erythrocytes, heart, kidney, lung and liver have virtually no detectable Thy-1 antigen². Two other antigenic determinants

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can be found on the rat Thy-1 molecule and these are recognised by xenogeneic (rabbit) antiserum and are referred to as the rat specific, and the rat-mouse cross reacting, xenoantigenic determinants^{2,14}. Similar antigenic determinants have been described in the mouse¹⁵.

There has been considerable confusion as to the chemical nature of the Thy-1 molecule with suggestions that it is a glycolipid^{16,17}, a protein of various sizes^{18,19} or a glycoprotein^{20,21} in mouse, and a protein or glycoprotein in rat²². Glycoproteins have been purified from rat thymocytes²³ and brain²⁴ which clearly carry all the Thy-1 antigenic determinants at high specific activities, and the conflicts between these results and the work of others is discussed below. The purifications involved the solubilisation of antigen in deoxycholate from crude membrane preparations, followed by gel filtration and affinity chromatography with columns of lentil lectin coupled to Sepharose-4B. The affinity column bound most of the brain Thy-1, but about 50% of thymocyte Thy-1 did not bind. This fraction was purified by an antibody column against brain Thy-1. Thus three Thy-1 preparations were obtained: brain Thy-1, thymocyte Thy-1 which binds to lentil lectin (Thy-1L+) and thymocyte Thy-1 which does not bind (Thy-1L-). The three preparations could not be distinguished by their antigenic determinants and both thymocyte and brain Thy-1 were of similar immunogenicity in rabbits. In contrast, slight differences in mobility of the glycoproteins were noted after electrophoresis on polyacrylamide gels in sodium dodecyl sulphate. On 12.5% polyacrylamide gels, brain Thy-1 had an apparent molecular weight of 24,000, while for thymocyte Thy-1L+ and Thy-1L- the values were 25,000 and 27,000, respectively^{23,24}.

We describe here the results of amino acid and carbohydrate analysis of the Thy-1 glycoproteins and also report experiments to determine the nature of the antigenic determinants.

Preparation of Thy-1 samples for chemical analysis

The three forms of Thy-1 were purified from rat thymocytes and brain as before^{23,24} (except that twice recrystallised sodium deoxycholate was used) and the minor impurities in thymocyte Thy-1L- were removed by gel filtration on a column of Sephadex G-200 in 0.5% sodium deoxycholate. The purified fractions contained a large amount of deoxycholate after concentration by ultrafiltration, and this was removed by precipitation of the glycoprotein with ethanol in which deoxycholate is soluble (M. J. Crumpton, personal communication). Ethanol was added to a final concentration of 75% together with a few drops of saturated sodium acetate in ethanol (to improve flocculation of the precipitate) and the mixture was left for 48 h at -20 °C. The precipitate was recovered and washed in absolute ethanol by centrifugation at 1,800 *g* for 30 min at -10 °C. The precipitates were readily soluble in water, and a small amount of turbidity was removed by centrifugation at 100,000 *g* for 30 min. The resulting pellets contained less than 5% of the Thy-1 by antigenic activity or amino acid and carbohydrate analysis, and probably consisted of trace impurities from the deoxycholate.

This precipitation gave yields of 70% for both thymocyte forms and 40% for Thy-1 from brain. The procedure had no effect on the antigenicity of the glycoproteins whose specific activities for Thy-1.1 and Thy-1 xenoantigenic determinants were similar to those of the pure preparations before precipitation^{23,24}. The electrophoretic behaviour of the glycoproteins on polyacrylamide gels in sodium dodecyl sulphate (SDS) was also unaffected by the precipitation, and, as Fig. 1 shows, none of the three preparations showed detectable contaminant bands when stained for protein or carbohydrate.

Preliminary studies on the size of Thy-1 in the absence

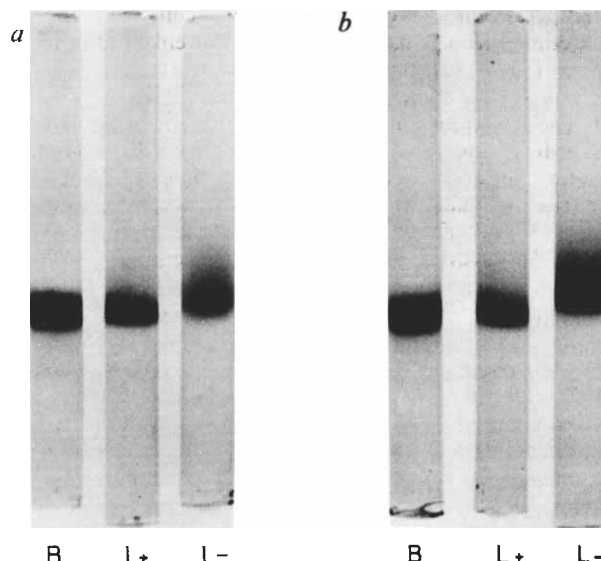


Fig. 1 Purity of Thy-1 fractions analysed by polyacrylamide gel electrophoresis in sodium dodecyl sulphate. B, L+ and L- denote Thy-1 purified from brain, and Thy-1L+ and Thy-1L- from thymocytes respectively, staining for protein with Coomassie blue (a) or for carbohydrate with periodic acid-Schiff (b) stain²³. The Thy-1 glycoproteins which had been precipitated with ethanol and solubilised in water, were electrophoresed on 7.5% polyacrylamide gels in SDS²³. 14 μ g and 22 μ g of the glycoproteins were loaded for gels stained for protein and carbohydrate, respectively.

of deoxycholate have been carried out by sedimentation equilibrium methods in the ultracentrifuge, and most Thy-1 was in an aggregate of 250,000–350,000 weight-average molecular weight (unpublished results of P. Kuchel). After addition of deoxycholate, Thy-1 was found in an antigen-detergent complex of molecular weight 29,000, in agreement with previous studies^{22,24}. Aggregation in the absence of detergent would be expected for a membrane molecule which was normally associated with lipids.

Table 1 Amino acid composition of Thy-1 glycoproteins

	Brain Thy-1	Thymocyte Thy-1L+ Thy-1L-	
Asx	12.7	12.6	13.0
Glx	9.1	9.1	9.1
His	4.1	3.8	4.0
Lys	6.9	7.2	7.0
Arg	7.5	7.5	7.2
Thr	7.6	8.6	8.3
Ser	7.4	7.1	7.9
Pro	3.6	3.8	3.0
Ala	3.3	2.9	3.2
Cys	3.1	3.2	3.2
Gly	6.0	4.9	5.0
Tyr	2.0	2.0	2.0
Val	7.3	7.4	7.4
Ile	3.9	4.1	4.0
Leu	10.4	11.2	11.1
Phe	4.0	3.7	3.8
Met	1.1	0.9	0.8

Amino acid analyses are expressed as the mean number of each amino acid residue per 100 residues and are the result of at least four analyses in each case. For brain Thy-1 and thymocyte Thy-1L+, two completely independent preparations of each were analysed, while for thymocyte Thy-1L- one preparation was used. The amino acids are arranged into hydrophilic, intermediate and hydrophobic groups²⁵ reading down the table. Samples for amino acid analyses were hydrolysed with constant boiling HCl at 110 °C for 20 h and analysed on a Locarte or modified Beckman analyser²⁶. A correction for the destruction of threonine and serine was calculated from 24- and 72-h hydrolyses of Thy-1 from brain and applied to all analyses. Cysteine was estimated as cysteic acid after performic oxidation²⁷. The s.e.m. was <5% of each mean value except for proline values (<12%).

Chemical analysis of the Thy-1 glycoproteins

The preparations of Thy-1 shown in Fig. 1 were analysed for their amino acid (Table 1) and carbohydrate compositions (Table 2) by standard procedures. The amino acid analyses were very similar for the three forms of Thy-1 and the only differences which were significant after statistical analysis (not shown) were those between the leucine and glycine content of brain Thy-1 in comparison with thymocyte Thy-1. The compositions were so similar, however, that any differences which may exist in the protein part of the molecules will only be clearly revealed by amino acid sequence analysis. If the amino acids are grouped on the basis of their hydrophobicity (Table 1), it can be seen that Thy-1 is much less hydrophobic than some membrane proteins²⁵ and indeed is notable for its large proportion of hydrophilic residues. Hydrophobicity in amino acid compositions is, however, not an invariable feature of membrane proteins and the major human red cell glycoprotein which is superficially similar to Thy-1 is also quite hydrophilic in its amino acid composition³². In detail the amino acid compositions of the red cell glycoprotein and Thy-1 are very dissimilar.

If the molecular weight of 25,000 as determined by electrophoresis on polyacrylamide gels in SDS is taken as correct, then the size of the polypeptide portion would be about 17,000 and the values in Table 1 should be multiplied by 1.5 to give the number of residues per molecule.

In contrast to the amino acid analyses, marked differences in carbohydrate composition were found particularly between brain and thymocyte Thy-1 with smaller differences between thymocyte Thy-1L+ and Thy-1L-. These results are shown in Table 2 and are expressed as residues per 100 residues of amino acids. If the figures are multiplied by 1.5, an estimate of residues per molecule can be obtained. Galactosamine was found only in brain samples and no trace was detected in thymocyte Thy-1. In contrast, sialic acid was present in very small amounts in brain Thy-1, but in much larger amounts in thymocyte Thy-1. The amounts of fucose, glucose and galactose also differed by a factor of 2 or more between brain and thymocyte Thy-1, while smaller differences on a percentage basis were found between mannose and glucosamine contents. Given that differences in composition are likely to reflect much larger differences in structure, the carbohydrate chains of brain and thymocyte Thy-1 may be completely unrelated.

Between thymocyte Thy-1L+ and Thy-1L- the compositions were much more similar, but differences in the amount of galactose, mannose, glucosamine and sialic acid may be significant. These differences in composition do not provide a simple answer as to why Thy-1L- fails to be bound by the lentil lectin, except to reinforce the view that it is likely to be due to differences in the carbohydrate rather than the polypeptide structure. The differences between Thy-1L+ and Thy-1L- are typical of the microheterogeneity of carbohydrate residues observed for many glycoproteins³³. The large differences between brain Thy-1 and thymocyte Thy-1, however, represent a much more unusual order of heterogeneity of carbohydrates and could reflect a similar polypeptide chain carrying unrelated carbohydrate structures in the two tissues.

In the carbohydrate analysis by gas-liquid chromatography only small amounts of material other than known sugars were found; these included traces of lipid (see legend to Table 2). The procedures used in the purification of Thy-1 involving deoxycholate and ethanol would be expected to remove non-covalently-bound lipids and previously no lipid-bound phosphorus (less than 0.05 mg of phospholipid per mg of Thy-1) had been detected in brain Thy-1 (ref. 24).

From a summation of the amino acid and carbohydrate analyses, the percentage composition by weight of the molecules was calculated (Table 2). The values for carbo-

Table 2 Carbohydrate composition of Thy-1 glycoproteins

Carbohydrate residues per 100 amino acid residues Analyses by gas-liquid chromatography	Thymocyte		
	Brain Thy-1	Thy-1L+	Thy-1L-
Fucose	1.8	1.0	0.9
Mannose	11.9	10.6	9.4
Galactose	1.8	5.5	6.9
Glucose	0.6	1.3	1.1
Glucosamine	6.3	7.0	8.3
Galactosamine	1.0	0.0	0.0
Sialic acid	0.2	1.8	2.2
Amino sugars by ion-exchange chromatography			
Glucosamine	8.3	9.4	11.7
Galactosamine	1.0	0.0	0.0
Sialic acid by fluorescent method			
Sialic acid	0.3	2.1	2.9
Percentage by weight of carbohydrate	29%	32%	35%

The carbohydrate analysis of the three glycoproteins is expressed as the number of carbohydrate residues per 100 amino acid residues (Table 1). The results shown are the means of four analyses for brain Thy-1 and thymocyte Thy-1L+ (on two separate preparations of each) and two analyses for thymocyte Thy-1L-. Neutral sugars, amino sugars and sialic acid were determined by gas-liquid chromatography after methanolysis and trimethylsilylation^{29,30}. Some minor unidentified peaks were observed in these analyses; one ran together with glucosamine and was thought to be stearic acid. Thus subsequent analyses were extracted with hexane after hydrolysis³⁰, and gas-liquid chromatography analysis of the hexane extracts confirmed the presence of a peak running in an identical manner to stearic acid. An upper limit to the amount of lipid present was estimated as 1.6, 1.3 and 0.9 lipid residues per 100 amino acids for Thy-1L-, Thy-1L+ and brain Thy-1, respectively. The remaining unidentified peaks amounted to less than 3% of the total area of the sugar peaks. Amino sugars were also estimated by ion-exchange chromatography after hydrolysis in 3 M *p*-toluene sulphonic acid, 0.2% tryptamine at 110 °C for 72 h (ref. 28). A correction for loss during hydrolysis was based on a 70% yield obtained for ovomucoid treated similarly. Sialic acid was also determined by a fluorescent method³¹, using 3,5-diaminobenzoic acid, on a Perkin Elmer MPF-2A fluorescence spectrometer. The interference of hexoses in these conditions (approximately 5% that of sialic acid) was measured in every assay and a correction made on the basis of the hexose composition shown above. For each value the s.e.m. was <12% of the mean.

hydrate content of about 30% are higher than for many membrane glycoproteins.

Chemical basis of Thy-1 antigenic determinants

Rat Thy-1, regardless of its tissue origin, carries at least three sets of antigenic determinants. These include Thy-1.1 which is recognised by mouse alloantiserum^{9,12} and two xenoantigenic determinants recognised by rabbit serum^{2,14}. The similarity between the amino acid compositions, and the differences in carbohydrate compositions of Thy-1 from brain and thymocytes suggest that these antigenic determinants are defined by the protein rather than the carbohydrate structure. To investigate this point further the susceptibility of the antigenicity to heating and proteolysis was studied. Both of these procedures should destroy protein determinants which are usually dependent on conformation³⁴ but leave carbohydrate antigenic determinants intact³⁵.

Samples of pure Thy-1 were heated for 10 min at various temperatures and assayed for Thy-1.1 and Thy-1 xenoantigenic determinants (Thy-1 rat specific and rat-mouse cross reacting xenoantigenic determinants were not distinguished). Figure 2 shows a typical experiment in which the activity of these antigenic determinants of brain Thy-1 was destroyed in the temperature range 70–80 °C. Similar results were obtained in experiments with thymocyte Thy-1L+.

The effects of proteolytic digestion were evaluated by measuring the loss of antigenic activity with the inhibition assays, and destruction of the polypeptide chain by SDS-polyacrylamide gel electrophoresis. Thy-1 was quite resistant to all the proteolytic enzymes tested, but after incubation

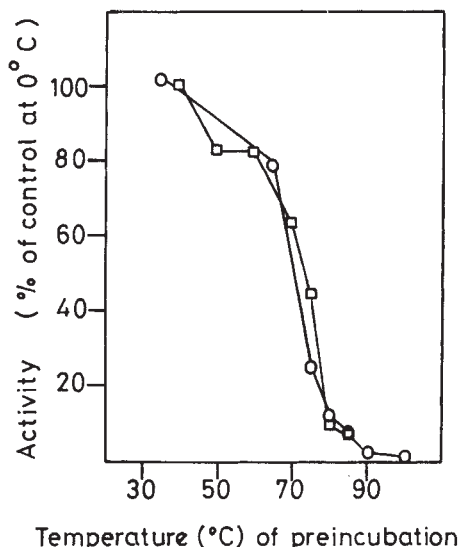


Fig. 2 Effect of heat on the Thy-1 antigenic determinants. The figure shows the results of two experiments on heating of brain Thy-1, assaying for Thy-1.1 (○) and Thy-1 xenoantigenic activities (□). Purified Thy-1 (approximately $10 \mu\text{g ml}^{-1}$) was heated for 10 min at the temperature shown in 0.1% sodium deoxycholate, 0.25% bovine serum albumin, 0.02% NaN_3 and 0.01M Tris-HCl, pH 8.0. The samples were cooled to 0°C and assayed for Thy-1.1 and Thy-1 xenoantigenic activities²³. These activities are expressed as the percentage activity compared with a similar sample kept at 0°C throughout (100%).

with Pronase for 24 h at 37°C , 80–95% of the antigenic activity of all Thy-1 determinants was destroyed (Table 3). Also the polypeptide chain was almost completely degraded as shown for brain Thy-1 and thymocyte Thy-1L+ in Fig. 3a and b, respectively. Pronase digestion for 3 h caused only partial loss of antigenic activity and partial removal of the polypeptide chain. When brain or thymocyte Thy-1 was incubated for 24 h with trypsin, chymotrypsin or papain (conditions as for Pronase in Table 3 except for the addition of cysteine HCl with papain), there was only partial loss (30–80%) of antigenic activity and the Thy-1 molecule. On a qualitative basis no discrepancies were observed between the amount of antigenic activity and the amount of Thy-1 band remaining on the polyacrylamide gel.

These results, together with the chemical analysis (Tables 1 and 2), and the experiments on the effect of heating, strongly (although not conclusively) suggest that all the antigenic determinants so far identified on rat Thy-1 are expressed by the protein part of the molecule.

Confusion about the nature of the Thy-1 molecule

There has been considerable disagreement about the chemical nature of the Thy-1 molecule. Most other workers have studied mouse Thy-1 but the basic characteristics of the molecule are likely to be similar in both mouse and rat.

Table 3 Effect of Pronase on Thy-1 antigenic activities

	Residual antigenic activities (%)	
	Thy-1.1 antigenic activity	Thy-1 xenoantigenic activity
Thy-1 (brain)	3, 7, 6	5, 6, 4
Thy-1L+ (thymocyte)	16, 10, 11	14, 9, 8

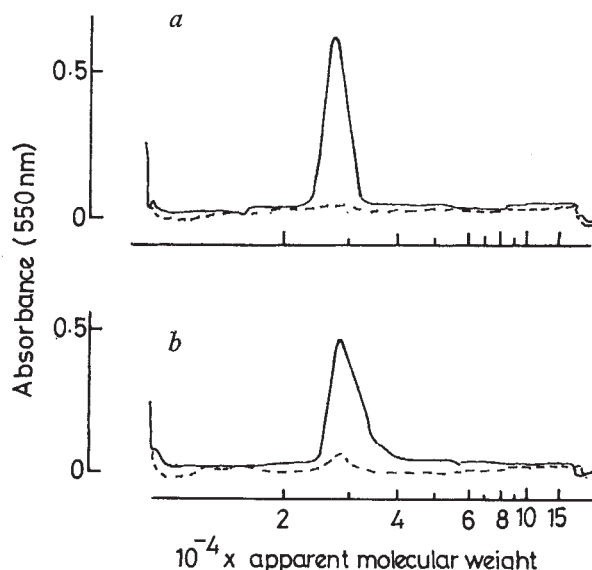
Purified brain Thy-1 or thymocyte Thy-1L+ was incubated at 37°C for 24 h in the presence or absence of Pronase. The antigenic activities of the digested and control preparations were then assayed and are shown for three independent experiments as the percentage of the digested material compared with the control incubation. In the incubation Thy-1 was at $400 \mu\text{g ml}^{-1}$ in 0.25% Na deoxycholate, 0.02% NaN_3 and 0.01 M Tris-HCl, pH 8.0, and Pronase was added at a weight ratio of 1 : 12 compared with antigen. The assay of antigenic activity was done at 0°C in the presence of a 1,500-fold excess of bovine serum albumin to inhibit residual Pronase activity.

A glycoprotein band similar to rat Thy-1 can be identified in mouse thymocyte membrane, and the mouse Thy-1.1 antigenic activity is found in the same place as rat Thy-1 after gel filtration in deoxycholate²³. Also Trowbridge *et al.*, using hyperimmune serum to rat brain Thy-1, could immunoprecipitate a glycoprotein of molecular weight about 25,000 from mouse thymocytes which had been surface labelled with ^{125}I and solubilised in detergent²⁰. This molecule was present in tissue culture cell lines which displayed Thy-1 antigen, but absent from mutants which had become negative for the antigen²¹. Using conventional anti-(Thy-1) alloantiserum they could not precipitate the 25,000 molecular weight glycoprotein and this confirmed the observations of Vitetta *et al.*¹⁶. The latter authors interpreted this to mean that Thy-1 antigens were inactivated by detergent, but this was clearly shown to be incorrect in studies using inhibition of radioimmunoassays²². It is more likely that the failure to precipitate Thy-1 was due to low affinity of conventional alloantiserum, leading to dissociation of antigen-antibody complexes in the washing of the immunoprecipitates. In similar experiments Atwell *et al.*¹⁸ immunoprecipitated a protein of apparent molecular weight 60,000. This result has not been confirmed by others, and may have been due to the use of nonspecific antiserum.

Sausser *et al.* have suggested that the Thy-1.2 alloantigen is associated with a protein of molecular weight about 40,000 which they obtained in preparations purified 60-fold for the antigen¹⁹. Thy-1 glycoprotein in the rat was pure when Thy-1.1 antigenic activity was purified 260-fold²³ and thus it is possible that the protein of Sausser *et al.*¹⁹ is a major contaminant in a fraction which also contains Thy-1 glycoprotein.

Miller and Esselman have suggested that mouse Thy-1 antigen is a ganglioside¹⁷ and this is completely inconsistent with the antigenic determinants being in the protein part of a glycoprotein. Their inhibitions of Thy-1 cytotoxicity assays with gangliosides, however, do not show the high degree of specificity expected for the Thy-1 alloantigens as defined by Reif and Allen⁴. Thus Miller and Esselman suggest that GM_1 ganglioside carries the Thy-1.2 determinant, yet ganglioside from a Thy-1.1 strain inhibits a

Fig. 3 Effect of Pronase on the Thy-1 glycoproteins. The Thy-1 glycoproteins were digested with Pronase as described in Table 3 and the effects analysed by electrophoresis on 7.5% polyacrylamide gels in SDS (as in Fig. 1). Equal amounts of protein from each digest and its control (about $5 \mu\text{g}$) were electrophoresed and after staining for protein with Coomassie blue the gels were scanned at 550 nm ²³. a, Scans for brain Thy-1 control (—) and brain Thy-1 after Pronase digestion (---). b, Thymocyte Thy-1L+ control (—) and Thy-1L+ after Pronase digestion (---).



cytotoxicity assay for Thy-1.2, 50% as well as ganglioside from a Thy-1.2 mouse¹⁷. Also, very large amounts of ganglioside were needed for inhibition of the cytotoxicity assay for Thy-1.2, for example for 50% inhibition approximately 500 ng of GM₁ (molecular weight 1,500) was required, compared with 10 ng of Thy-1 glycoprotein (molecular weight 25,000) for 50% inhibition of radioactive binding assays for Thy-1.1^{23,24}. Finally, other authors have not been able to find Thy-1 antigenic activity in lipid fractions^{36,37}.

Trowbridge *et al.* agree that Thy-1 is carried by a glycoprotein²⁰, but have suggested that the antigenic determinants of mouse Thy-1 are on the carbohydrate and not the protein part of the molecule²¹. This view was based on studies on mutant cell lines, but there is no obvious reason why the data cannot be interpreted equally well with the antigenic determinants carried by the protein part of the molecule.

Possible function for Thy-1 in thymus and brain

In mouse, peripheral T cells have Thy-1 and bone marrow cells do not⁷, whereas in the rat the converse is true¹³. Thus it is possible that Thy-1 is not of functional significance in these cell types of either species. In thymus and brain, Thy-1 is a major membrane glycoprotein and these are likely to be the tissues in which the Thy-1 molecules have a biological function.

A large part of the protein of the Thy-1 molecule is likely to be exposed at the cell surface since its amino acid composition is hydrophilic and the antigenic determinants are clearly available on intact cells. By analogy with other membranes, the carbohydrate structures are also likely to be exposed³⁸. Although there is no evidence about the function of Thy-1, the molecular properties do provide some limitations. For example, if the polypeptide chain mediates the specific function of Thy-1, the functions are presumably the same in both thymus and brain. Alternatively, the specific function may be mediated by the carbohydrate structures. In this case the polypeptide chain may anchor the molecule in the membrane and provide a backbone for the display of different carbohydrate ligands in thymus and brain. Cell surface carbohydrate has been suggested to be involved in cell-cell interactions by providing recognition sites for glycosyl transferases or lectins on other cells³⁸⁻⁴⁰. These ideas have gained particular emphasis in studies on slime moulds where strain specific lectins have been identified. These are found at the cell surface and may be involved in the aggregation of single cells in the slime mould's life cycle^{40,41}. In thymocytes and brain, Thy-1 would be an obvious possibility as a molecule involved in functions of this type. If this is the case, the differences in

carbohydrate composition between brain and thymus Thy-1 may reflect differences in the specificities of recognition in the two tissues.

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Nucleotide rigidity

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It is shown that in aqueous solution the backbone conformation of adenosine is as much flexible as that of 3'-AMP, 5'-AMP and 3', 5'-ADP indicating that nucleotides are not any more rigid than nucleosides. The flexible conformation of the monomeric components is conserved in the nucleotidyl units of destacked ApA, ApApA and poly(A), but it is not conserved in base stacked conditions. The findings are extended to guanosine, uridine and cytidine systems. It is projected that in aqueous solution, conformations of the individual nucleotidyl units of yeast tRNA^{Phe} are confined

to the classically stable domains in the base stacked region and non-rigid flexible structures populate in the unstacked region comprising D16, D17, G20, U47 and A76.

MANY investigations have been carried out on the conformation of mononucleotides in order to understand better the conformation of biologically functional nucleic acids. It has been proposed that the conformation of mononucleotides is rigid, and because of this their conformation is conserved in polynucleotides^{1,2}. The essential elements of the concept of a conforma-

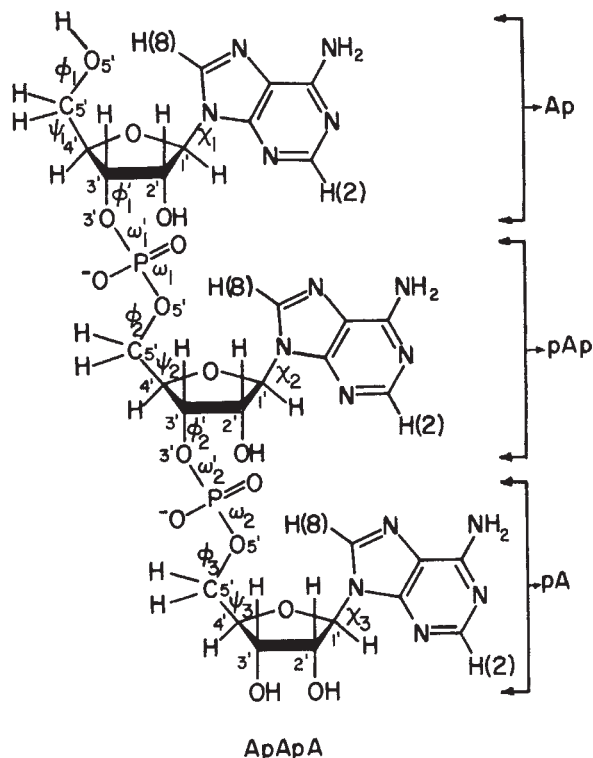


Fig. 1 Structure of ApApA, showing the atom numbering scheme and notations for the torsion angles (IUPAC-IUB recommendations).

tionally rigid nucleotide¹ are (1) that the nucleotides are conformationally more rigid than the corresponding nucleosides and that the phosphate group imparts rigidity to the nucleotides (this has been termed the 'phosphate effect'^{1,2}) and (2) the polymers achieve their biologically functional structure by rotation around the phosphodiester bonds. Quantum mechanical calculations suggested that the intrinsic conformational rigidity of a nucleotide may not be fundamentally different from that of the corresponding nucleoside³. The concept of rigidity is based on the finding that in the solid state nucleosides exhibit a constant conformational pattern—glycosidic torsion = *anti*; sugar pucker = ³E; $\psi \approx 60^\circ$ and $\phi \approx 180^\circ$ —which is absent in nucleosides^{1,2}. Recent X-ray crystallographic studies have found several nucleotides with conformations different from that of a rigid nucleotide⁴⁻⁹. Some differences have been found between solid state and solution state conformations¹⁰⁻¹². We have undertaken a systematic study of the fine conformational features in aqueous solution of adenosine (A), 3'-AMP

(Ap), 5'-AMP (pA), 3',5'-ADP (pAp), ApA, ApApA (Fig. 1) and poly(A) as well as uridine, 3'-UMP (Up), 5'-UMP (pU), UpU and poly(U) using high frequency nuclear magnetic resonance (NMR) spectroscopy combined with a Karplus-type analysis of the coupling constants. Here we examine the results obtained with respect to the rigid nucleotide concept.

All materials used were commercial preparations. ¹H NMR spectra were recorded at 100, 270 or 300 MHz in the Fourier transform mode using systems described elsewhere¹³⁻¹⁵. All NMR spectra were analysed using a UNIVAC 1108 computer and LACON III. The observed and simulated spectra of ApApA is shown in Fig. 2 and the data are summarised in Table 1.

Conformation in destacked oligomer and polymer

The backbone linkage is comprised of the C4'-C5'-O5'-P-O3'-C3' bond network. The conformation of the C4'-C5' and C5'-O5' bonds shows itself in the coupling constant sums $\Sigma(J_{H4'-H5'} + J_{H4'-P5'})$ and $\Sigma'(J_{H5'-P5'} - J_{H5'-O5'})$ respectively¹⁸. The near identity in the values of Σ (Table 1, Fig. 2) for each of the adenylyl parts in mostly destacked ApApA (72 °C) clearly shows the strong similarity in the time-average conformation about the three C4'-C5' bonds. The Σ data in Table 1 further reveal that this C4'-C5' bond conformation is very similar to that in mostly destacked ApA (72 °C) as well as to that in 3',5'-ADP, 3'-AMP, 5'-AMP and adenosine. Likewise, the Σ' data in Table 1 reveal that the conformational preference about the C5'-O5' bonds in destacked ApApA and ApA is strongly similar to that of the same bonds in 5'-AMP and 3',5'-ADP. The observed values of Σ and Σ' can be translated into an estimate of the percentage population of conformers using empirical equations developed in this laboratory¹⁸. These equations have been recently modified¹⁹. Computation of the population distribution of conformers shows that the *gauche-gauche* ($\psi, \psi_1, \psi_2, \psi_3 = 60^\circ$) population is 70-80%, and the combined population of *gauche-trans* ($\psi, \psi_1, \psi_2, \psi_3 = 180^\circ$) and *trans-gauche* ($\psi, \psi_1, \psi_2, \psi_3 = 300^\circ$) conformers is 20-30% irrespective of whether one is dealing with a nucleoside, 3'-nucleotide, 5'-nucleotide, 3',5'-diphosphonucleoside or a destacked dinucleoside monophosphate or a trinucleoside diphosphate. The *gauche'-gauche'* ($\phi, \phi_1, \phi_2, \phi_3 = 180^\circ$) population about C5'-O5' for all these compounds lies between 65-75%. The observed time-average preference for the *gauche-gauche* and *gauche'-gauche'* arrangement is further substantiated by the 1.5-1.8-Hz magnitude of the four-bond $^4J_{H4'-P5'}$ coupling²⁰.

Information on the conformation about the C3'-O3' part of the backbone can be obtained from the magnitude of the coupling constant $J_{H3'-P3'}$ (refs 15 and 21). The observed

Table 1 Coupling constant data for the various nucleic acid systems of the adenosine family

Temperature (°C)	Adenosine		3'-AMP		5'-AMP		5'-AMP		3',5'-ADP		ApA		ApA		ApApA*			ApApA*			poly(A)		poly(A)†	
	22	22	22	86	22	22	22	22	22	22	Ap- 12	pA- 12	Ap- 72	pA- 72	Ap- 18	pAp- 18	pA- 18	Ap- 72	pAp- 72	pA- 72	22	86	22	86
Solvent	D ₂ O	D ₂ O	D ₂ O	DMSO + D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	D ₂ O	DMSO + D ₂ O	DMSO + D ₂ O
H1'-H2'	6.2	6.2	5.7	5.8	5.5	3.6	4.0	4.8	4.6	3.4	2.8	3.5	4.9	5.0	4.8	2	5.9							
H2'-H3'	5.2	5.2	5.2	5.2	4.7	5.2	5.3	5.2	5.1	5.2	5.3	5.2	5.1	5.2	5.2	5.2	5.2							
H3'-H4'	3.5	2.9	3.7	3.5	3.7	5.5	5.8	4.4	4.6	4.4	4.6	4.4	4.6	(4.4)‡	4.7	4.9								
H3'-P3'	-	7.9	-	-	8.5	7.6	-	8.8	-	8.8	-	-	-	(7.5)‡	7.9	-	-							
H4'-H5'	2.8	2.4	3.1	3.3	2.9	2.5	2.8	2.6	2.8	2.6	2.8	2.6	2.8	2.8	2.7	2.8								
H4'-H5''	3.6	3.3	3.1	3.9	2.9	3.5	3.7	4.0	4.2	3.9	3.8	3.8		3.9	3.8	3.8								
Σ	6.4	5.7	6.2	7.2	5.8	6.0	6.5	6.6	7.0	6.7	6.5	6.6		6.7	6.5	6.6								
H4'-P5'	-	-	1.7	1.5	2.2	-	2.0	-	1.8	-	1.8	1.8		-	1.8	1.8								
H5'-P5'	-	-	5.0	5.7	5.3	-	3.0	-	4.9	-	4.9	5.0		-	4.8	5.0								
H5''-P5'	-	-	5.0	5.6	5.3	-	3.3	-	4.9	-	4.9	5.0		-	4.6	5.0								
Σ'	-	-	10.0	11.3	10.6	-	6.3	-	9.8	-	9.4	10.0		-	9.4	10.0								
H5'-H5''	-12.6	-11.7	-12.0	-12.0	-11.6	-13.0	-12.0	-12.8	-11.6	-12.8	-11.8	-12.0		-12.8	-11.8	-12.0								

Concentration is 0.02 M for ApA and ApApA, pD 7.0. Concentration is 0.05 M for the mononucleotides, pD 5.4. The pD is such that the phosphate will be mostly monoanionic like the oligomers and polymers. Data for the monomers at 22 °C can be compared with data for oligomers and polymers at higher temperatures because at the concentration used, the monomer conformations are not significantly perturbed in the temperature range. See, for example, data for 5'-AMP at 22 °C in D₂O and at 86 °C in DMSO-D₂O. Error in coupling constants ± 0.1 Hz unless otherwise stated.

* The assignments for the three 1' resonances proposed by Kondo *et al.*¹⁶ has been verified by the incremental methods of Tso *et al.*¹⁷ by comparing ApA, ApApA and ApApApA.

† Poly(A) dissolved in 50% d₆-DMSO and 50% D₂O at 86 °C, 0.05 M in mononucleotide. For error in the analyses see legend of Fig. 4.

‡ These values are approximate because of the presence of extensive second order perturbations at the frequency used of 270 MHz.

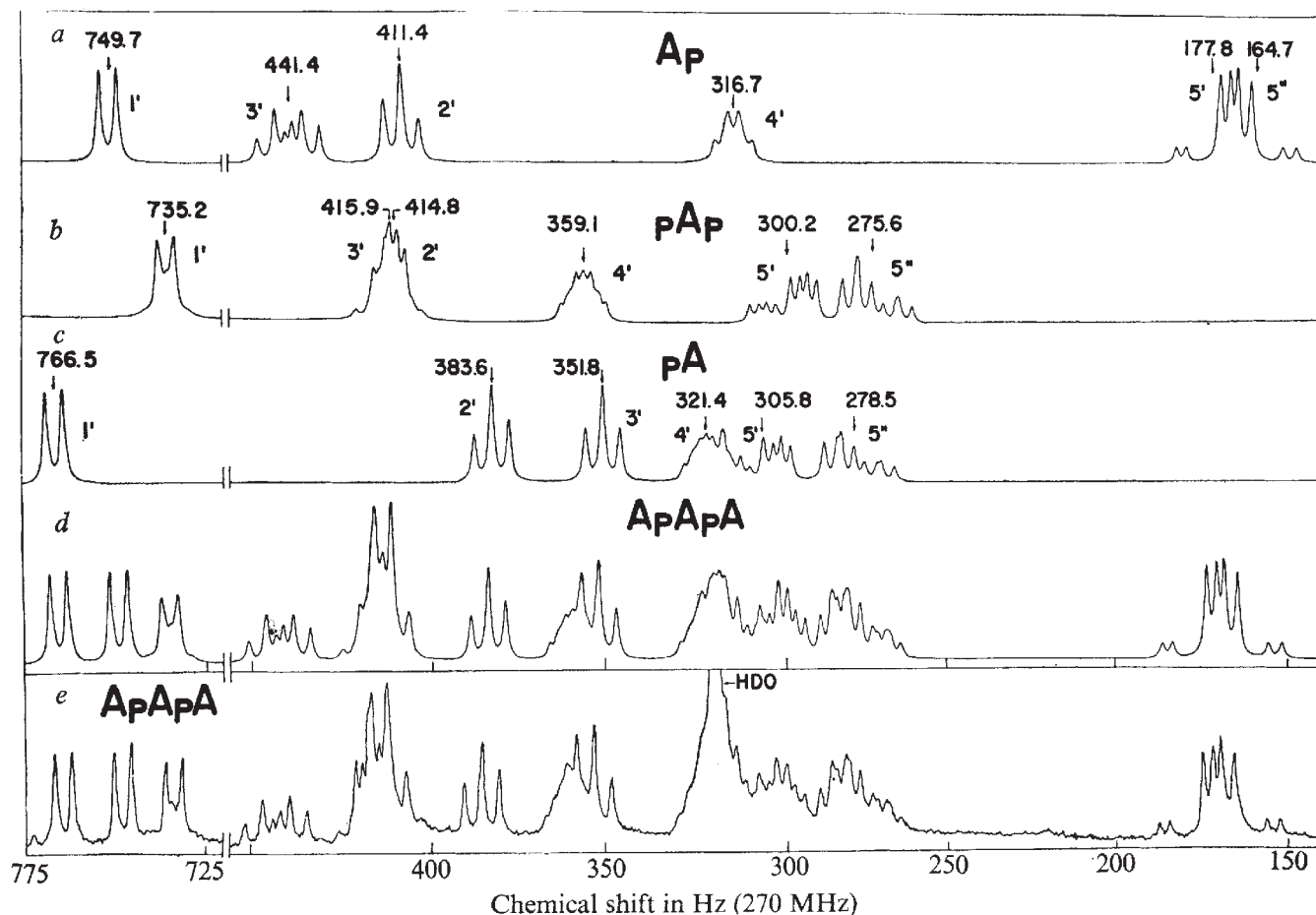


Fig. 2 *a, b, c*, Computer simulations of the Ap-, pAp- and pA parts of ApApA. *d*, Combination of the above three parts into one to produce ApApA simulation. *e*, The 270 MHz ^1H experimental NMR spectrum of ApApA at 72 °C, pH 7.0, 0.02 M.

values of $J_{\text{H3'-P3'}}$ indicate that in the component monomers, as well as the dimer and trimer at 72 °C, the C3'-O3' bonds have similar time average conformations.

The ribofuranose ring conformation in nucleic acids and their components in aqueous solution may be treated as a simple C2'-endo (^3E) and C3'-endo (^3E) equilibrium^{22,23} or as an equilibrium of entities with precise phase angles and amplitudes of pucker²⁴. The pseudorotational approach²⁴ is a major improvement over the classical description for solid state data. However, for the majority of nucleotide systems in aqueous solution the pseudorotation approach may not be sufficiently accurate to be an improvement over the simple qualitative approach of a $^3\text{E} \rightleftharpoons ^3\text{E}$ equilibrium^{23,25}. The percentage population of ^3E conformers in adenosine, 3'-AMP, 5'-AMP, 3',5'-ADP, ApA 72 °C, ApApA 72 °C were computed (Fig. 3*a*) and the data show that the component monomers, irrespective of whether a nucleoside or a nucleotide, have the same time-average conformation for the ribose ring. These are only slightly different from that of ApA 72 °C and ApApA 72 °C. Later discussion will show that the ApA and ApApA ribose rings did not approach the same conformational distribution of the components because at the temperature used (72 °C both ApA and ApApA display a certain degree of stacking in D₂O (refs 27, 28).

Two conclusions emerge from the data: (1) In the adenosine series, in aqueous solution the nucleoside and the nucleotides have the same time-average conformation with respect to the ribose ring, the C5'-O5', C4'-C5' and C3'-O3' bonds. It does not seem therefore, that this part of the nucleotide backbone is conformationally more rigid than in nucleosides, at least for the system investigated. The observation that the phosphate group present at the 3' or 5' position or at both the positions

has no detectable effect on the population distribution of conformers about C4'-C5', C5'-O5', C3'-O3' and the ribose ring argues against an effect of the phosphate group on conformation. The mononucleotide components 3'-AMP, 5'-AMP and 3',5'-ADP essentially conserve their backbone conformation as they become integrated into the framework of the destacked oligomers ApA and ApApA.

It is of interest to determine whether these conclusions can be extended to the case of a polymer such as poly(A). The spectrum of poly(A) in the destacking solvent DMSO/D₂O at 86 °C was taken and the entire spectrum was computer simulated (Fig. 4). The extracted values for Σ , Σ' and $J_{\text{H3'-P3'}}$ (Table 1) as well as the computed percentage of ^3E population (Fig. 3*a*) clearly reveal that destacked poly(A) has a time-average backbone conformation very similar to that of the component monomeric units.

Conformation in the stacked oligomer and polymer

The discussion presented shows that the monomeric units conserve their preferred conformation for the backbone in the destacked oligomer and polymer. This is not true in the case of stacked molecules. Comparison of the computed percentage populations of ^3E conformers for stacked poly(A), ApApA and ApA (low temperature in D₂O) with that for the component monomers (Fig. 3*b*) reveals that the ribose ring in the mostly stacked oligomers and polymer displays a conformational preference dramatically different from that of the monomeric components. The ^3E populations in the monomers are about 40%, while the corresponding populations for ApA, ApApA and poly(A) in conditions which favour stacking lie in the

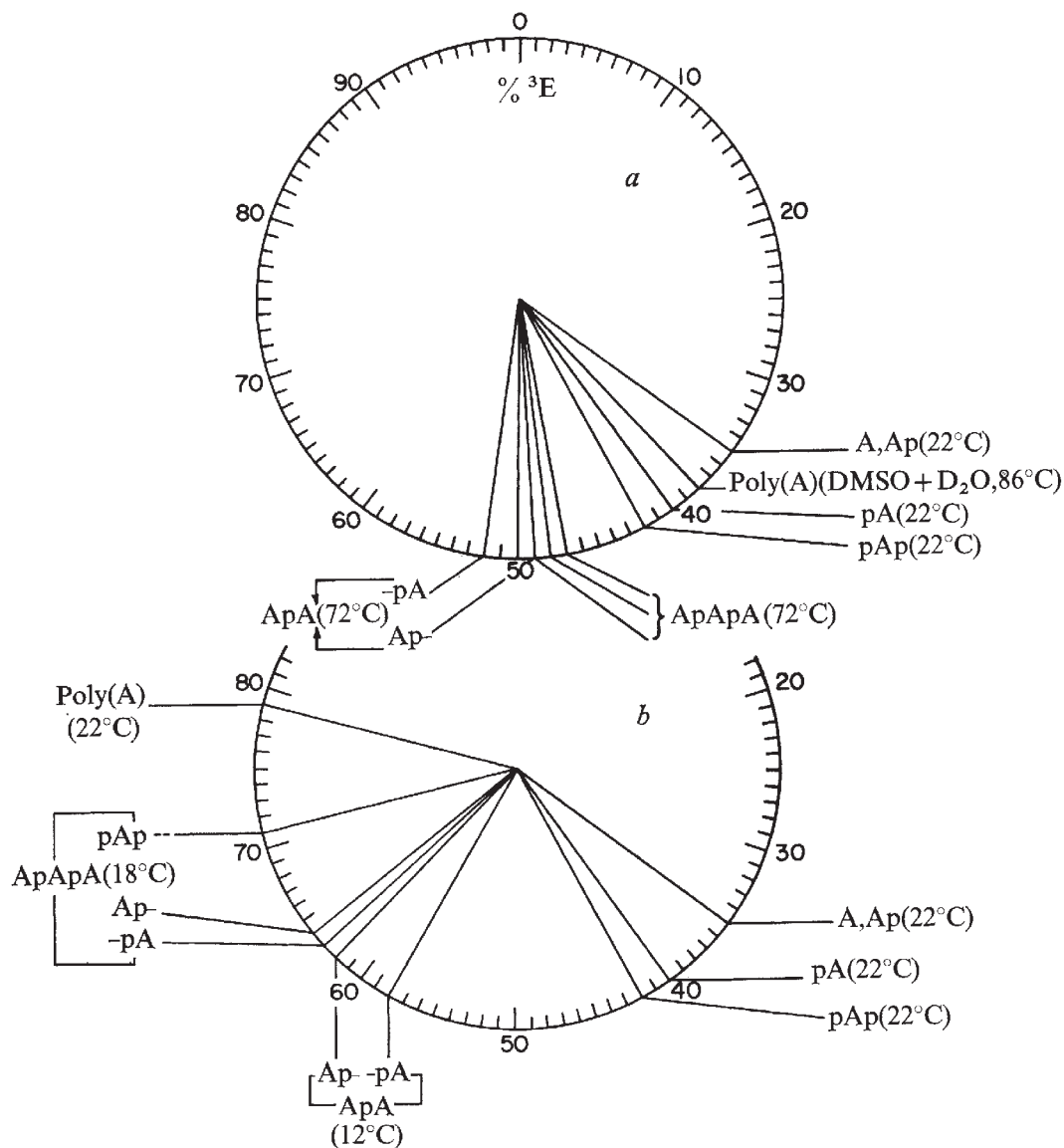


Fig. 3 *a*, Computed percentage populations of the 3E ribose conformers in monomers and in mostly destacked ApA, ApApA and poly(A). *b*, Computed percentage populations of the 3E ribose conformers in monomers and in mostly stacked ApA, ApApA and poly(A). The populations are computed by the method described elsewhere (ref. 15, and F. S. Ezra, C. H. Lee, N. S. Kondo, S. S. Danyluk, and R.H.S.). This method enables us to calculate $\% ^3E$ or $\% ^2E$ populations from either $J_{3'4'}$ or $J_{1'2'}$ using an empirical sum of $J_{1'2'} + J_{3'4'} = 9.5$ Hz. This sum was derived from 30 ribose units of 15 different dinucleoside monophosphates (ref. 15, and F. S. Ezra, C. H. Lee, N. S. Kondo, S. S. Danyluk, and R.H.S.). In the crystals of ApApA, the ribose assumes 3E pucker²⁶. The abbreviations A, Ap pA and pAp refer to adenosine, 3'-AMP, 5'-AMP and 3', 5'-ADP. The percentage population of 3E conformer = $100 - \% ^2E$.

range of 60–80%. The higher end of the range is preferred by the central –pAp– unit of ApApA and the nucleotidyl units in poly(A). It should be stressed that as the monomeric components become integrated into the framework of a biologically functional stacked polymer, not only is there a significant increase in the populations of 3E conformers, but also there is a shift in the kind of pucker they prefer, that is, the monomers prefer 2E sugar pucker ($\approx 60\%$) but the oligomers and polymer prefer 3E sugar pucker. Mononucleotides do not conserve their isolated individual conformations as they become part of a polymer in the case of base-stacked adenylyl polynucleotides. The low temperature ApApA and poly(A) spectra are extremely complex and so cannot be completely analysed to obtain the values for $J_{H3'-P3'}$, Σ and Σ' . However inspection of the data in Table 1 for 3'-AMP, 5'-AMP, 3', 5'-ADP and ApA (12°C) shows that stacking significantly changes the conformational preference about the C5'-O5' bond of the –pA unit. On the basis of the temperature dependence of Σ and Σ' values in ApA, we have previously suggested that the C4'-C5' and C5'-O5' bonds are predominantly *gauche-gauche* in the totally base-stacked state²⁹.

Conformation in oligo- and polynucleotides other than the adenosine family

Published ribose coupling constant data^{12,30,31} on guanosine, 3'-GMP and 5'-GMP in aqueous solution clearly reveal that the nucleosides and the nucleotides display similar distribution of 2E and 3E conformers. The Σ values^{12,30,31} show that in all three cases the percentage population of the *gauche-gauche* ($\psi = 60^\circ$) conformer about C4'-C5' is about 70–75%. Unusual line broadening complications prevent the analyses of the 1H NMR spectra of GpG and GpGpG and one cannot at present determine the conformational status of the guanosine nucleotidyl units in the oligomer.

Single-stranded polypurine nucleotides are known to exhibit considerably more base stacking than poly(U) in aqueous solution. The coupling constant data for 3'-UMP, 5'-UMP, UpU and poly(U) (Table 2) are, within error limits, similar in their magnitudes, which indicates that in aqueous solution at room temperature the backbone conformation of poly(U) is as flexible as that of the constituent mononucleotides, unlike the case in the adenylyl series discussed earlier.

Table 2 Coupling constant data for the various nucleic acid systems of the uridine family

Coupling nuclei	Uridine	3'-UMP	5'-UMP	UpU		poly(U)*
H1'-H2'	4.4	5.0	4.8	4.2	3.9	5.5
H2'-H3'	5.3	5.2	5.0	5.2	5.1	5.5
H3'-H4'	5.5	5.2	4.3	5.3	5.0	4.0
H3'-P3'	—	8.2	—	8.2	—	9.0
H4'-H5'	3.0	2.8	2.4	2.4	2.4	—
H4'-H5"	4.4	4.2	2.8	4.0	3.2	—
Σ	7.4	7.0	5.2	6.4	5.7	7.5
H4'-P5'	—	—	2.1	—	2.0	—
H5'-P5'	—	—	4.3	—	4.4	—
H5'-P5"	—	—	5.1	—	4.5	—
Σ	—	—	9.4	—	8.9	—
H5'-H5"	-12.7	-12.8	-11.7	-13.1	-11.9	-12.5

The concentration is 0.05 M for the monomers. The pD of 3'-UMP and 5'-UMP solutions were kept at pD 5.4 so that the phosphate will be mostly monoanionic like the oligomers and polymer at pD 7.4. For UpU concentration 0.02–0.03 M, pD 7.4. The temperature in all cases was 22 °C. Error in coupling constants ± 0.1 Hz unless otherwise stated.

*From ref. 32.

Comparison of the values of $J_{H3'-H4'}$ in uridine and 5'-UMP indicates that this coupling constant undergoes a reduction of 1.2 Hz on phosphorylation at the 5' position. This translates into an increase of about 15% in the population of 3E conformers for the ribose ring on 5' phosphorylation. Equally interesting is the observation that the value of Σ decreases by 2.2 Hz on 5' phosphorylation of uridine, indicating that the presence of the phosphate group increases the *gauche-gauche* ($\psi = 60^\circ$) populations about C4'-C5' by a substantial 20–25%. These observations in the uridine series are very different from those of the adenosine and guanosine series and agree with the projections of a conformationally rigid nucleotide^{1,2}. Comparison of the coupling constant data for cytidine, 3'-CMP, 5'-CMP and CpC in Lee *et al.*¹⁵ indicate that in the cytidine series, as in the uridine series, the 5' nucleotide is conformationally more rigid than the nucleoside; but unlike the uridine system, the mononucleotide conformation is not conserved in the dimer.

Relevance to tRNA conformation

From the foregoing discussion it is clear that the conformational properties of common nucleic acid components, oligo- and

polynucleotides in aqueous solution are not governed by a common principle such as the concept of a conformationally rigid nucleotide^{1,2}. On the one hand in the adenosine and guanosine series, the backbones of the nucleosides and nucleotides are equally flexible, but on the other hand in the uridine and cytidine series, the nucleotides are relatively less flexible than their corresponding nucleosides. The concept that the conformational destiny of mononucleotides, as they become part of polynucleotides is ordained by their intrinsic conformations and that they maintain their isolated conformations in the polymer, is true when significant amounts of the polymer are in the destacked state. This is analogous to the solution conformations of coenzyme A and nucleoside diphosphohexoses which exist as a blend of mostly unfolded and folded conformers^{14,21}. The conformational conservation breaks down as soon as the mononucleotides become integrated into the backbone framework of a biologically functional base-stacked polynucleotide. The nucleotidyl units in a base-stacked oligomer and polymer exhibit backbone conformations significantly different from the individual conformations of the monomeric components in aqueous solution.

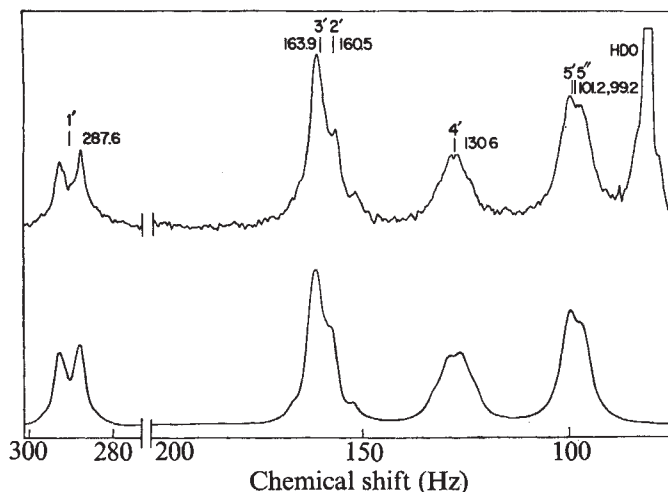
Even though our findings seem to support the reported presence of non-rigid nucleotides in tRNA³³, we wish to examine our results with regard to the sophisticated structure of yeast phenylalanine tRNA derived by Sussman and Kim³⁴ by examination of the common features from three sets of atomic coordinates. These authors report the presence of extensive base stacking along the central core of the two molecular axes, that is, the amino acid arm and T ψ C arm make one axis and the dihydrouridine arm and the anticodon arm the other. The bases that are not stacked are D16, D17, G20, U47 and A76. Assuming that the structure of yeast tRNA^{Phe} in crystals is the same as that in solution^{35–38} from the present findings one may project that in the fully base-stacked regions of tRNA^{Phe} in aqueous solutions, the sugar-base torsion will occupy the *anti* domain, the ribose ring a 3E pucker, the C3'-O3', C4'-C5', C5'-O5', O3'-P and O5'-P preferring domains centred around $\phi' \approx 210^\circ$, $\psi \approx 60^\circ$, $\phi \approx 180^\circ$, $\omega' \approx 300^\circ$ ($\approx 60^\circ$) and $\omega \approx 300^\circ$ ($\approx 60^\circ$). The present data do not enable us to make a distinction between right-handed stacks ($\omega'/\omega = 300^\circ/300^\circ$) and left-handed loop stacks ($\omega'/\omega = 60^\circ/60^\circ$), even though $\omega'/\omega = 60^\circ/60^\circ$ stacks are expected only in the loop region. One would expect the unstacked region comprising³⁴ D16, D17, G20, U47 and A76 to show conformational freedom and flexibility so much so that alternative conformers such as 2E , $\psi \approx 180^\circ/300^\circ$ become allowed. Because of the demonstrated conformational nexus in aqueous solution among sugar-base torsion, sugar-pucker, C3'-O3' torsion and ω'/ω rotations (refs 15, 39; F. S. Ezra, C. H. Lee, N. S. Kondo, S. S. Danyluk, and R. H. S., unpublished, and D. M. Cheng and R. H. S., unpublished) one would also anticipate that those fractional populations which exist in 2E conformations in the unstacked region will also populate in domains in which $\omega'/\omega \approx 180^\circ/300^\circ$, $\phi' \approx 270^\circ$ and the value of χ , though still in the *anti* domain, several degrees above the ones encountered in the base stacked region. Our general conclusion is that in aqueous solution, conformations of the individual nucleotidyl units of yeast tRNA^{Phe} are confined to the classically stable domains, as has been reported in crystals by Sussman and Kim³⁴ and that non-rigid flexible structures are confined to that region of tRNA which is not base stacked.

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Fig. 4 100 MHz Fourier transformed 1H -(^{31}P) spectrum of poly(A) in 50% d_6 -DMSO and 50% D_2O at 86 °C, 0.05 M (top) and the computer simulation (bottom). The extracted coupling constants from simulation are only accurate ± 0.5 Hz because of line broadening complications. It should be pointed out that the accuracy would be considerably less if phosphorus decoupling had not been used.



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letters to nature

Detection of hot gas in clusters of galaxies by observation of the microwave background radiation

THE Uhuru (ref. 1) and the Ariel V (ref. 2) satellites have shown that many rich clusters are powerful sources of X rays. This emission has been interpreted either as thermal bremsstrahlung of very hot gas filling the clusters^{3,4}, or as inverse Compton scattering of photons by relativistic electrons^{5,6}. Spectral evidence⁷ has begun to favour a thermal origin for this radiation, implying the existence of large amounts of hot gas. Such gas would have a profound influence upon the dynamics of radio sources, and its mass might well be a substantial fraction of that of the whole cluster. Hot gas may therefore be a major constituent of the Universe, so that independent confirmation of its existence is extremely important. We present here observations of small diminutions in the cosmic microwave background radiation in the directions of several rich clusters of galaxies. This confirms the existence of large amounts of very hot gas in these clusters and indicates that their X radiation is thermal bremsstrahlung and not inverse Compton emission.

A necessary consequence of the existence of hot gas in clusters is the Compton scattering of the cosmic microwave background by hot electrons⁷. As a result, some of the microwave photons are redistributed with slightly higher energies, and the effect may be observed at radio frequencies as a small diminution in the background temperature. The magnitude of this effect is predicted to be $\sim 0.5 \times 10^{-3}$ K for the gas clouds associated with cluster X-ray sources ($\rho_{\text{gas}} \approx 5 \times 10^2$ atom m^{-3} , $T_{\text{gas}} \approx 2.5 \times 10^8$ K, $l \approx 300$ kpc (refs 4, 7)). Parijskij⁸ has claimed to have detected this effect at a slightly higher (1.2×10^{-3} K) level in the direction of the Coma cluster, but his result was marginal and in any case seems to have been implicitly withdrawn⁹.

We present the first results of an attempt to detect this effect, using the 25-m telescope at the Chilbolton Observatory of the SRC Appleton Laboratory. We have observed 7 clusters, of which 6 are known to be X-ray sources. The remaining cluster (A2218) is very distant, so that no strong limits to its X-ray flux are available. It was included because it is a very rich cluster (richness 4 on Abell's scale) and was known not to contain any non-thermal radio sources likely to mask a diminution in the intensity of the microwave background.

The Coma cluster (A1656) was included, although it is confused by the weak radio source 5C4.85 (36 ± 6 mJy at 10.6 GHz), for which a small correction has been made. Upper limits on the radio flux density from the other clusters are < 100 mJy at 1,400 MHz, though A478 is only 43' distant from the bright radio source 3C109 and no limit on its flux density has been published.

To minimise the effect of non-thermal background sources, it is desirable to observe at the highest possible frequency compatible with atmospheric stability. A frequency of 10.6 GHz was chosen, at which the Chilbolton aerial has a half-power beamwidth of 4.5'. Observations were made using a twin-beam system, the beams being separated on the sky by 14' in the azimuthal direction, which is just sufficient to take the reference beam beyond the central regions of the largest cluster (Coma). Ideally, the beams should be more widely separated than this, but atmospheric irregularities and distortion of the off-axis beam then become severe problems. The receiver included an uncooled one-stage parametric amplifier, having a noise temperature of ~ 280 K and a bandwidth of ~ 200 MHz. The output time constant was 1 s and samples were taken at 0.5-s intervals. The two beams were alternately directed at the centre of a candidate cluster for 20 s at a time; after a complete cycle the mean (x_i) and standard error (σ_i) of the 80 samples (~ 20 independent samples) were stored on-line in a Ferranti ARGUS 500 computer. This 'wagging' procedure was continued until, at the end of an allotted observation period (~ 4 h), the cumulative mean and s.d. were derived, weighting the individual observations by $1/(\sigma_i^2 + x_i^2/2)$. This latter term proved to be an effective gate against spurious high-significance measurements, caused by either atmospheric irregularities with time scales ~ 1 min, receiver drift, or occasional interference from radar. The system was calibrated at regular intervals by injecting a known noise signal into the main beam. The antenna temperatures measured in this way were then converted into equivalent sky temperatures, using radio sources of known flux density and the measured beamshape to determine the aerial efficiency (55%). We have also attempted observations using drift scans. This method is, however, particularly sensitive to atmospheric irregularities and was abandoned in favour of the wagging technique.

The results are given in Table 1. They represent a total of 674 h of observations in 12 weeks during 1975 Nov-1976 June. They show the temperature of background radiation in the directions of the centres¹⁰ of the 7 Abell clusters, relative to a

Table 1 Data obtained

Cluster	Temperature ($\pm$ s.d.) (10^{-3} K)	$\sqrt{\chi^2 - \nu} n - 1$
A376	-0.13 ± 0.66	1.75†
A478	$+0.33 \pm 0.52$	-1.09
A576	-0.71 ± 0.57	0.34
Coma*	-1.51 ± 0.40	1.89†
A2218	-1.94 ± 0.54	1.92†
A2319	-0.13 ± 0.41	0.38
A2666	-0.27 ± 0.35	0.42
Blank Sky	-0.01 ± 0.32	-0.39

*Combination of two different positions (see text).

†Slightly discordant.

peculiarly weighted mean of the temperature over arcs $14'$ on each side of the centre. Each value is the result of many observation periods, and statistical tests have shown that the data are generally concordant and conform rather well to the expected Gaussian distribution. All results have $\sqrt{\chi^2 - \nu} n - 1 < 2$, where n is the number of observation periods, so that considerable reliance can be placed on the final standard errors. The result for the Coma cluster is the weighted mean of two different positions, one of which is coincident with the centre of the cluster ($\alpha = 12$ h 57 min 24 s, $\delta = 28^\circ 15'$) and is only $3.16'$ from 5C4.85. A correction of $-1.35 \pm 0.25 \times 10^{-3}$ K has been calculated from the measured beamshape and flux density of 5C4.85 and applied to this result. No significant correction is required for the second position ($\alpha = 12$ h 57 min 37 s, $\delta = 29^\circ 13'$). For comparison, the uncorrected average value for coma is $-1.02 \pm 0.40 \times 10^{-3}$ K. The 'blank sky' observation was included to guard against the possible existence of systematic errors. The value shown is the weighted mean of three different positions, each chosen to have the same declination as either A576 or A2218, thereby ensuring that the observing conditions were nearly identical to those prevailing for the clusters themselves. No evidence of systematic errors was found. At our observing frequency, confusion from non-thermal background sources is expected to be $\sim 0.1 \times 10^{-3}$ K (J. V. Wall, personal communication).

It will be seen that, apart from A478, the results are in the expected sense and amount to a marginal detection of the expected diminutions at the predicted level of 0.5×10^{-3} K. The formal probability of obtaining results of this significance merely by chance is low, as may be seen from the value of χ^2 ($\chi^2 = 30.0$) for all 7 clusters assuming that the effect is absent. With 7 degrees of freedom, this indicates that the results are inconsistent with the assumption of zero mean at the 99.99% confidence level. This test takes no account, however, of the relevant fact that 6 out of 7 results are in the same sense. A further test is the value of the weighted mean of all the clusters, which is $-0.63 \pm 0.17 \times 10^{-3}$ K. It must be realised that this figure is only relevant as a test of the null hypothesis and has no particular astrophysical significance. It should also be emphasised that no data have been rejected and that Table 1 is a complete list of clusters for which we have results. Bearing this in mind, it is clear that the results are most encouraging, if not yet entirely conclusive. Observations with greater sensitivity are now proceeding, in an attempt to confirm the effect at a higher confidence level, and to measure the shape of the diminution for one cluster.

These observations therefore strongly suggest the presence of hot plasma ($> 10^8$ K) filling clusters of galaxies. It seems highly probable that the X radiation from these clusters is just the thermal radiation of this gas, and that previous inverse Compton interpretations can now be ruled out. Confirmation of these effects will also show directly that the microwave background radiation is truly cosmic, and must have its origin beyond distant clusters of galaxies.

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D. N. Matheson. We also thank Dr M. S. Longair and Professor Sir Martin Ryle for provoking this research, and the many members of the Mullard Radio Astronomy Observatory who helped with the observations.

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Visibility of galaxies

IT is well known that our counts of galaxies could be seriously biased by selection effects, largely determined by the brightness of the night sky. To illustrate this, suppose the Earth were situated near the centre of a giant elliptical galaxy, then the mean surface brightness of the sky would appear some 8–9 mag brighter than is observed from our position in the Galaxy (~ 23 V mag (arc s) $^{-2}$ looking toward the galactic pole, discounting atmospheric and zodiacal contributions^{1,2}). Optical astronomers would then find extragalactic space an empty void; spiral and irregular galaxies would be quite invisible and all they would easily detect of galaxies would be the core regions of ellipticals very similar to their own. They would be blinded to much of the Universe by the surface brightness of their parent

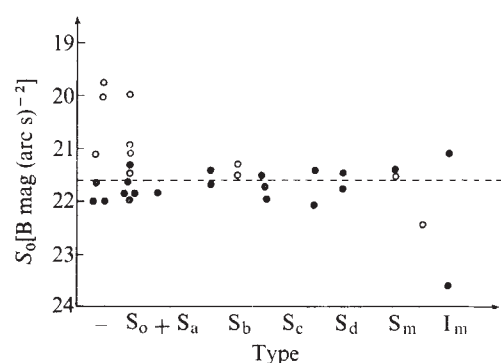


Fig. 1 Central surface brightnesses S_0 of exponential disks of spiral and irregular galaxies taken from Freeman² plotted against galactic type. Filled circles show most reliable data, open circles less reliable. Some of the original points, subsequently shown to be incorrect (K. C. Freeman, personal communication) have been removed. (— — —) is at 21.65 B mag (arc s) $^{-2}$.

galaxy. But this blinding is clearly a relative matter and we should ask to what extent we are blinded by the spiral galaxy in which we exist, faint as it may appear by comparison. I will argue that strong indirect evidence already exists that our knowledge of galaxies is heavily biased by the sky background, and that the true population of extra-galactic space may be very different from the one we can see.

Begin by recalling two sets of observations applying to spirals and ellipticals respectively, that have been in the literature some time. Both are based on de Vaucouleurs' discovery³ that the radial surface brightness distribution $\sigma(r)$ of galaxies (apart from second order effects like spiral arms) can be described by the law

$$\log_{10} \left[\frac{\sigma(r)}{\sigma(0)} \right] = - \left(\frac{r}{\alpha} \right)^{1/\beta} \quad (1)$$

where α is a scale-length and $\beta = 1$ for spirals and 4 for ellipticals. Using equation (1) one can show that the total luminosity L_T is

$$L_T \equiv L(r \rightarrow \infty) = \int_0^\infty 2\pi r \sigma(r) dr = \frac{(2\beta)!}{(\log_e 10)^{2\beta}} \pi \sigma(0) \alpha^2 \quad (2)$$

In 1970 Freeman² collated the best photometry then available for spiral and irregular systems and found the surprising result that $\sigma(0)$ was virtually constant for the disks of all but a few

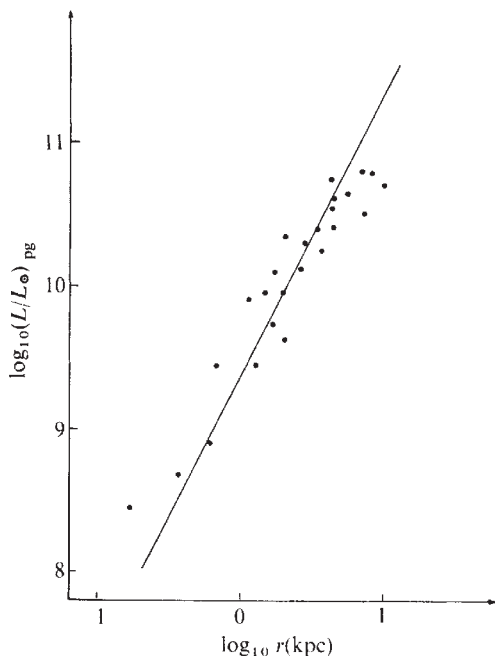


Fig. 2 Luminosities and radii of Fish's sample of ellipticals. Only those points obtained using equation (1) are shown. Fish obtained some other data using velocity dispersions. The line is the best fit, with a slope of 2.

galaxies in the sample. If we express $\sigma(0)$ in B mag (arc s)⁻² and then write it as S_0 , then Freeman finds $\langle S_0 \rangle_s = 21.65 \pm 0.3$ (see Fig. 1).

Fish⁴ had earlier discovered an apparently unrelated fact about a sample of well studied ellipticals: he showed that their binding energy Ω varied as $M^{3/2}$ where M is their mass. Fish actually measured L and α for his sample, and then assumed that ellipticals generally have the same mass to light ratio. Disentangling the directly measured quantities from the assumptions

$$\frac{\Omega}{M^{3/2}} = \text{const} \rightarrow \frac{GM^2}{RM^{3/2}} = \text{const} \rightarrow \frac{L_T^{1/2}}{R} = \text{const}$$

But R (the radius) $\sim \alpha$ so, using equation (2), Fish's law implies that

$$\frac{L_T}{\alpha^2} \sim \sigma(0) = \text{const}$$

for the ellipticals in his sample, which comprised most of the best studied individuals at that time. Fish's raw data, not plotted in his paper, are shown in Fig. 2, and we see that Fish's law is conceptually identical with Freeman's, and can be stated thus: well studied bright ellipticals have a central surface brightness $\langle S_0 \rangle_E = 14.80 \pm 0.9$ B mag (arc s)⁻².

When the two laws are placed contiguously in this fashion one begins to suspect that observational selection is responsible, though one is puzzled by the large discrepancy in $\langle S_0 \rangle_s - \langle S_0 \rangle_E = 6.85$ mag between the two galactic types. I now argue that observationally selection can indeed account for both Fish's and Freeman's results rather simply, and even supplies the two different values of $\langle S_0 \rangle$ with few assumptions beyond equation (1), which is well tested.

Fish's and Freeman's sample comprise the major proportion of galaxies for which careful surface photometry existed at that time. What criteria are used in selecting galaxies for this type of investigation? De Vaucouleurs⁵ has discussed this matter but there is no clearcut answer and, historically, apparent size, apparent area, apparent luminosity and surface brightness have probably all been important. The selection has, however usually, been based initially on photographic material. Because of the limited dynamic range of emulsions the real luminosity is not well measured, and to a first approximation at least, galaxies with large angular radii or areas will generally appear most luminous on a photographic plate. (The belated discovery of Zwicky's compacts, many of which are highly luminous, testifies to this natural selection effect.) Our hypothesis is that it is the apparent radius r_{ap} (and area r_{ap}^2) which gives a galaxy a spectacular appearance on a plate, and makes us believe that it is probably luminous and suitable for more detailed investigation. On this hypothesis the galaxies in the Fish-Freeman samples were therefore probably chosen, consciously or otherwise, mainly for their apparent radii and areas in photographic surveys.

For constant L_T and S_L (where S_L is the limiting surface brightness out to which diffuse objects are readily apparent on a photographic plate) intuition leads one to expect that r_{ap} will have a maximum as $\sigma(0)$ varies. If $\sigma(0)$ is low, even the central isophotes will lie close to σ_L and r_{ap} will be small; conversely, if $\sigma(0)$ is high, then by equation (2), α and hence r_{ap} will also be small.

If σ_L is the limiting surface brightness corresponding to S_L (where S_L is σ_L expressed in magnitudes) then equation (1) becomes

$$\log_{10} \left[\frac{\sigma_L}{\sigma(0)} \right] = - \left(\frac{r_{ap}}{\alpha} \right)^{1/\beta}$$

Then:

$$r_{ap} = \alpha(0.4)^\beta (S_L - S_0)^\beta \quad (3)$$

Using equation (2) to eliminate α from (3) we obtain

$$r_{ap} = K L_T^{1/2} \left[\frac{1}{\pi 2\beta!} \right]^{1/2} (0.4 \log_e 10)^\beta \exp(0.46 S_0) (S_L - S_0)^\beta \quad (4)$$

where K is a normalising constant whose value can be obtained from any well studied galaxy, elliptical or spiral. Using NGC3379 (refs 5, 6) with r_{ap} in kpc and L_T in units of $10^9 L_\odot$, $K = 1.20 \times 10^{-4}$ and the results are plotted in Fig. 3. As anticipated there are well defined maxima in r_{ap} for the two types of galaxy. What is surprising is that the $S_0(r_{ap} = \text{max})$ are separated for the spirals and ellipticals by 6.52 mag, which is in the right direction and

very close to the precise size required to explain both Fish and Freeman's laws. Moreover, since for most plate material of interest here, S_L must be close to $24 \text{ B mag (arc s)}^{-2}$, then $S_0(r_{ap} = \max) = 21.83$ for spirals and 15.31 for ellipticals, so the absolute values are close to those observed as well. Furthermore the scatter in the spiral $S_0(r_{ap} = \max)$ would be smaller than for ellipticals as the observations demand. There are no free parameters in Fig. 3 for the β follow from equation (1) and L_T changes only the vertical scale (see equation (4)). It is important to note that L_{ap} , where L_{ap} is the apparent photographic luminosity, also has a double peaked distribution similar to r_{ap} in Fig. 3, but the details are complex and for brevity we omit discussion of L_{ap} here.

What is one to make of the uncanny fit of Fish's and Freeman's data to equation (4)? Conservatively, one could argue that their data are not representative and that the fit is an unlikely coincidence, but a coincidence none the less. Though this could well be correct it is not an heuristically useful hypothesis and we pursue it no further here.

The alternative is to accept our hypothesis, and acknowledge that surprisingly powerful selection effects are involved in picking out galaxies which are apparently large and apparently luminous, and that only objects with a very narrow range of $\sigma(0)$ will be so selected. If this is true, and only further observations can prove it to be so, then it is likely that our present knowledge of the galactic luminosity function is seriously in error, in that galaxies of all luminosities with $\sigma(0)$ different from

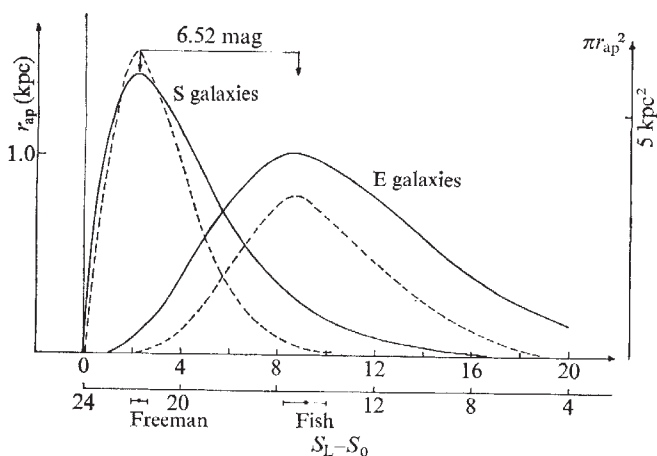


Fig. 3 Apparent radii r_{ap} (solid lines) and areas πr_{ap}^2 (dashed) for ellipticals and spiral disks of total luminosity $L_{pg} = 10^9 L_{\odot, pg}$, as a function of $(S_L - S_0)$. Radius scale on left, area on right. Changing L varies only the vertical scale. The scale under the abscissa is based on the assumption that $S_L = 24 \text{ B mag (arc s)}^{-2}$.

the Fish-Freeman values will be under-represented. Stated thus the hypothesis is hardly new. At one end of the scale Zwicky's work has revealed the hitherto unsuspected compacts while Arp⁸, for instance, has demonstrated that the $\log L/\log r$ plane is everywhere populated where it is detectable, and that there are no good reasons for believing that lower surface brightness objects, and parts of objects, do not exist. Fish's and Freeman's laws are then strong, but indirect evidence for this hypothesis.

On this hypothesis galaxies are like icebergs and what is seen above the sky background may be no reliable measure of what lies underneath. An apparently insignificant dwarf may be the core of a large galactic system of either type. The resolved Sculptor/Fornax systems have S_0 as low as $25 \text{ B mag (arc s)}^{-2}$ so the whole range of S_0 between 25 and 15 could be populated. Conceivably the missing mass in groups, clusters and the Universe may be accounted for in this way.

To test the selection hypothesis it will be necessary to move away from the narrowly selected range of objects with spectac-

ular apparent radii. In a given region of sky all objects suspected to be non-stellar will have to be studied in detail. Since galaxies often contain both elliptical and exponential components it will be necessary to measure both S_0 and α for each component before L_T can be established.

Such a systematic attack could take years. In the shorter term it is interesting to wonder if some few icebergs may be turned up by more *ad hoc* approaches:

(a) Figure 3 reveals that the most dramatic gradient in r_{ap} occurs for spirals with small $S_L - S_0$. The S.R.C. IIIaJ survey should have a deeper S_L than the IIa0 and in some cases faint spirals on the IIa0 should show a dramatic increase in r_{ap} in going to the IIIaJ.

(b) The cores of both spirals and ellipticals commonly obey the de Vaucouleurs elliptical law. Insignificant-looking ellipticals may sometimes be the tips of giant low- $\sigma(0)$ spirals which should therefore contain hydrogen. A quick 21-cm survey of a large number of dE might be interesting since Fisher and Tulley's sample⁹ contains few dE (dwarf ellipticals).

(c) Although the spiral and H II regions of a galaxy generally represent only a small fraction of the total light, they do have a higher contrast against the night sky. Deep Schmidt plates at wavelengths designed to enhance this contrast (for example H α) may show some spiral arms with no underlying disk. Ring galaxies may be objects of this type.

(d) Some systems show signs of tidal interference with no apparent cause. 'Dwarfs' in the vicinity warrant careful examination.

(e) Galaxies are often a composite of the two types. Take a disk with $S_0 = 21.65 \text{ mag}$ and add to it an elliptical component with the same S_0 and L_T ; equation (4) gives the elliptical r_{ap} as 7% of the disk r_{ap} and so the core will appear insignificant, though it represents the tip of a massive halo. It may be possible to detect such halos by careful measurement of the core scale-length.

(f) Double radio sources with 'empty fields' may sometimes have at their centre a giant galaxy of low S_0 , which may be detectable with careful photometry.

In opposition to the selection hypothesis in its most radical form we should point out that $\sigma(0)$ is not likely to be independent of the total mass, M , of the galaxy, and the giants (that is high mass objects) will in general have a higher $\sigma(0)$ and will not be so easily missed. But if we suppose that M and $\bar{\rho}$ are the important physical parameters for classifying galaxies then on dimensional grounds $\sigma(0) \sim M^{1/3} \bar{\rho}^{2/3}$. $\sigma(0)$ for a giant of $10^{11} M_{\odot}$ will then in general be less than 3 magnitudes higher than for an extreme dwarf of $10^8 M_{\odot}$ which is a small difference compared to the total observed range of S_0 between ~ 8 for extreme compacts¹⁰ and 25 for Sculptor-Fornax systems, so this is probably not an important effect.

Observations are urgently required to determine how important surface brightness selection may be.

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Trends in the climate of the North Atlantic Ocean over the past century

THE dominant large scale phenomena determining the climate of the North Atlantic Ocean are the current systems of the Gulf Stream and the North Atlantic Drift as well as the pattern of atmospheric circulation associated with the semi-permanent centres, the Iceland low and the Azores high. Recently, three long time series of data have become available which throw some light on the interactions between these ocean-atmosphere systems and which appear to indicate the importance of advective processes, as opposed to upwelling, in determining long term variations in the surface temperature of the ocean.

Interactions between the ocean currents and the atmospheric circulation have been studied by Brooks¹ and subsequently by Bjerknes². The latter postulated that long-term fluctuations in the sea-surface temperature of the North Atlantic were determined by such interactions and, in particular, that periods of low sea-surface temperature corresponded with periods of intensification of the Iceland low and the Azores high and, hence, of the mid-latitude westerly winds. The increased wind was assumed to produce an intensification of the flow of the Gulf Stream. Bjerknes considered that a drop in surface temperature of the northern North Atlantic may be attributed to upwelling

produced by increased cyclonic wind-drag and also to an increase in the cold winds from North America. Working with data from the North Atlantic weather stations from 1952 to 1965, Rodewald³ has confirmed that cooling of the surface waters of the ocean is associated with an intensification of the atmospheric circulation.

The three long-time series of data referred to above are:

(1) Data for sea-surface temperature, based largely on observations from ships in transit, are held in the marine data deck of the US National Climatic Centre. Monthly means of sea-surface temperature for each $5^\circ \times 5^\circ$ quarter of Marsden squares 145 and 182 (see Fig. 1) have been made available to the author by the Pacific Environmental Group of the US National Marine Fisheries Service. These areas of the western approaches to Great Britain were selected because they have been well covered by ships and virtually complete monthly data are available from 1854 to 1968, updated to 1973 by data supplied by the UK Meteorological Office.

(2) As an index of changes in the strength of flow of the Gulf Stream, Martin⁴ has recently presented a time series of data for mean sea level at points along the coast of Florida, for the period 1902–64.

(3) Cohen and Sweetser⁵ have provided a time series of frequencies of tropical cyclones in the Atlantic for 1871–1973. The positions and intensities of the semi-permanent centres reflect the conditions for the formation and movement of hurricanes and Namias⁶ has shown that a southward

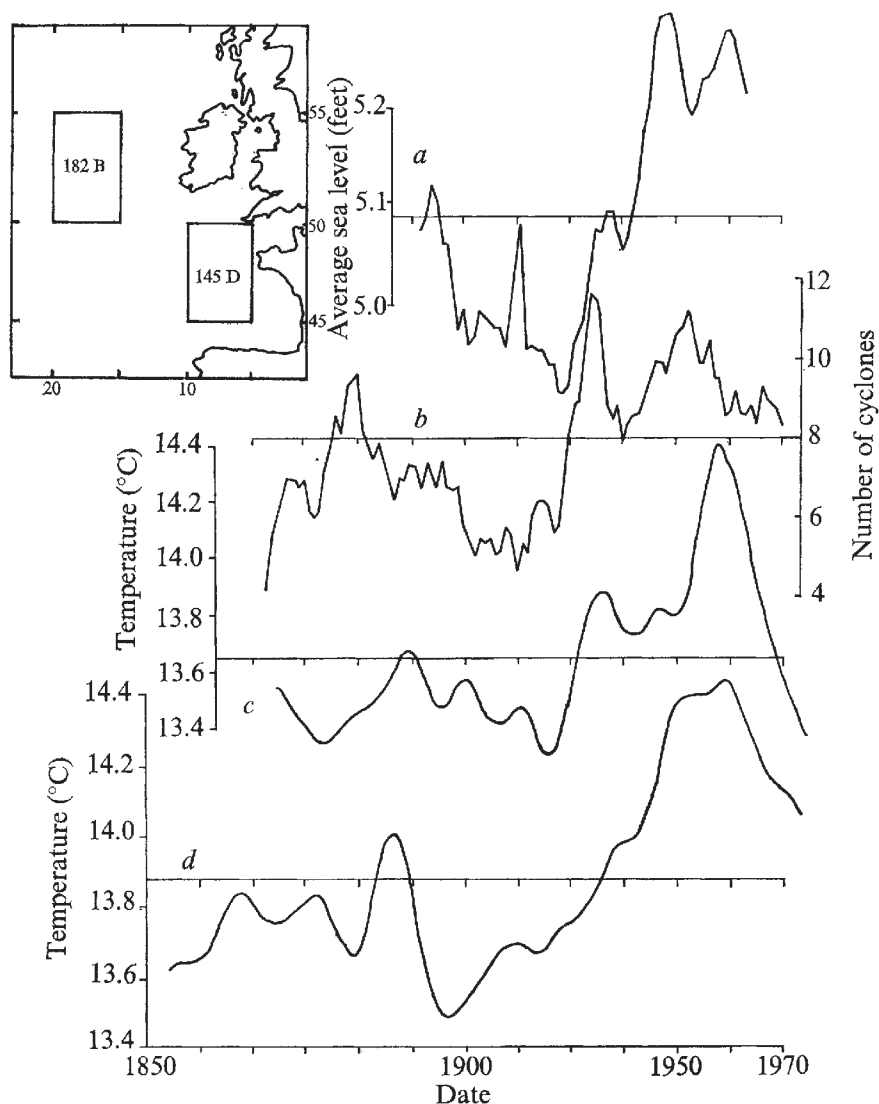


Fig. 1 *a*, 5-yr running mean of average sea level at stations along the coast of Florida, redrawn from Martin⁴. *b*, 7-yr running mean number of Atlantic tropical cyclones, redrawn from Cohen and Sweetser⁵. *c*, Smoothed variations of sea-surface temperature for Marsden square 182B (50–55°N, 15–20°W). *d*, Smoothed variations of sea-surface temperature for Marsden square 145D (45–50°N, 5–10°W). The variables in *c* and *d* were both smoothed by the application of eigenvector filters which, in both instances, were approximately equivalent to 7-yr running means. Inset shows position of Marsden squares 145 and 182.

shift of the Azores high is associated with diminished hurricane activity. It follows that the frequency of tropical cyclones can be used as a guide to the long term changes in the intensity of atmospheric circulation over the North Atlantic.

These three time series are illustrated in Fig. 1, all expressed as smoothed variables (see legend). The general similarities between the long term patterns of change are obvious, showing that low sea-surface temperatures in the North Atlantic are associated with a higher-than-normal flow in the Gulf Stream and a lower-than-normal frequency of tropical cyclones, indicating an above average intensity of the mid-latitude westerlies. Martin illustrated the relationship between Gulf Stream flow and sea-surface temperature in an area off the west coast of Scotland (data from Smed⁷) and he considered that this supported the proposal by Iselin⁸ that strong flow in the Gulf Stream leads to a retention of warm water in the North Atlantic Gyre and a reduction in the flow of this water in the North Atlantic Drift, with a consequent drop in temperature. This conflicts with Bjerknes' interpretation that cooling is caused by upwelling induced by increased cyclonic wind-drag.

If the Martin-Iselin interpretation, based on advected changes, is correct, then one would expect the cooling and warming trends, measured at the sea surface, to be more pronounced during the winter and spring in the absence of vertical temperature stratification, while cooling by upwelling should be equally or even more pronounced during periods of summer stratification.

Examination of the time series of sea-surface temperature data for individual months in Marsden squares 182 and 145 indicates that, while a common long-term pattern of change is present in each month of the year, it was clearest in the winter and spring months, whereas during stratification (Schroeder⁹) it tends to be obscured by higher frequency variations, presumably because of local fluctuations in radiation. Thus, these data clearly support the hypothesis that temperature change is produced by advective processes as opposed to upwelling.

It would appear that the time series illustrated in Fig. 1 provides a description, in relative terms, of the major climatic elements of the North Atlantic Ocean covering the past hundred years. It must be stressed that these series contain little or no evidence on which to base predictions; moreover, there are shorter-term phenomena of equal or even greater magnitude which influence both the atmospheric circulation and sea temperatures. Nevertheless, it is of interest to compare the long term history of variations in sea-surface temperature with the more recent changes. Wahl and Bryson¹⁰, on the basis of Rodewald's studies of data from the ocean weather stations, state that "it seems that we have experienced, in the past 29 yr or so, a change in Atlantic Ocean temperatures which amount, in the Gulf Stream vicinity, to about one-sixth of the difference between total glaciation and our present climate"; they also mention two other recent changes with equivalent magnitudes: the northward penetration of the monsoon rains in West Africa and a global increase in average snow and ice cover. The temperature series presented in Fig. 1 suggest that, although there has been a marked drop in temperature in the north-eastern Atlantic, it still has some way to go before attaining values similar to those for the 1920s and there is no evidence from any of the variables that the recent changes are in any way different from those of similar, or even larger, magnitude that have occurred during the past hundred years.

An approximately 10-yr periodicity in sea-surface temperature variations has been reported for several areas of the North Atlantic by Maximov¹¹ (based on analyses of data presented by Smed⁷ for the period 1900-1960) and also by Southward *et al.*¹², for a station in the English Channel from the early 1920s. Both reports draw attention to a possible

relationship with the 10-11 yr cycle in the numbers of sunspots.

The sea-surface temperature data for Marsden squares 182 and 145 have been analysed by a filter technique based on the calculation of eigenvectors of the matrix of serial correlations for a specified maximum lag. These analyses showed that the dominant systematic pattern of variation of the residual time series, following the removal of the long term trends contains considerable 10-11-yr periodicity which tends to be in phase with the sunspot cycle, with high temperature anomalies corresponding with sunspot maxima.

The recent work of Parker¹³ on pressure distributions in January and July for the period 1750-1958 clearly associates a deepening of the Iceland low in January with the descending phase of the sunspot cycle. This is supported, in general terms, by the data presented by Lawrence¹⁴ of pressure distributions for the month of June for the last eight sunspot cycles (1880-1960). The phase relationships are somewhat confused but the most likely implied association is between a deep Iceland low and positive temperature anomalies. This appears to conflict with the general pattern of intense westerlies and low temperatures. In this case, however, the deepening of the Iceland low is associated with positive pressure anomalies in the area of the Barents Sea and therefore with an increase in southerly anomaly winds over the North Sea and at least some way out over the north-eastern Atlantic. This seems likely to account for the positive relationship between sea-surface temperature and sunspots at the English Channel station and may well apply to areas further out in the Atlantic.

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Mantle composition derived from the chemistry of ultramafic lavas

ESTIMATES of the composition of the Earth's mantle have been based on both geochemical and geophysical studies (refs 1-3). As might be expected, there are some discrepancies between the estimates particularly for Na₂O and TiO₂. Such discrepancies in part arise from uncertainties concerning the degrees of melting which many volcanics represent and, possibly, the restricted tectonic settings of the ultramafic nodules studied. High MgO basalts and ultramafic lavas, some of which have been termed komatiites⁴ occur among the Archaean volcanic rocks. They represent exceptionally high degrees of melting (up to 50% or more) of peridotite source regions. Potentially such volcanics may be used to make a precise estimate of the composition of their mantle source region. High MgO lavas are well preserved in the Archaean Belingwe greenstone belt of southern Rhodesia, where they form part of a sequence deposited

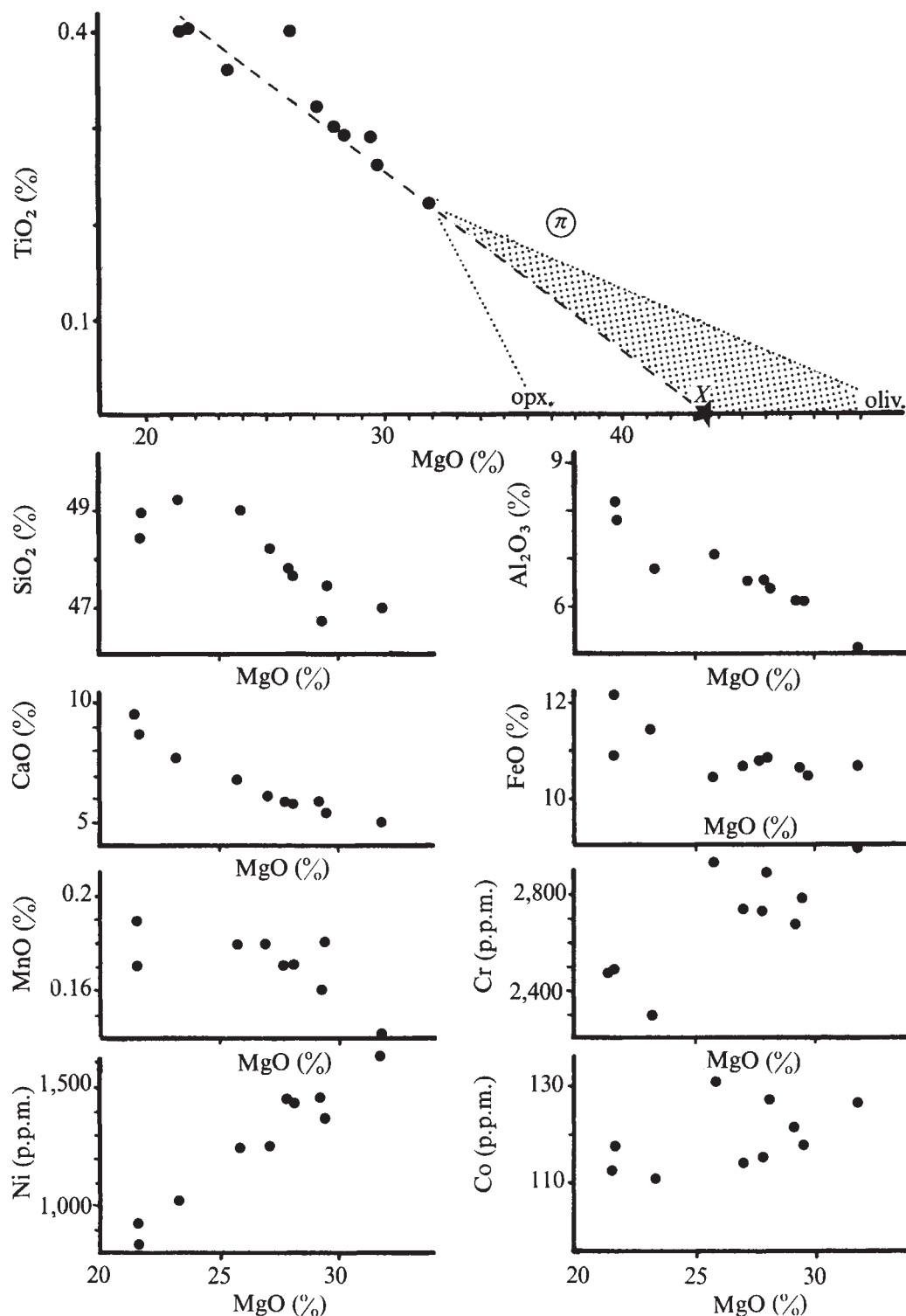


Fig. 1 Major and trace element compositions of ultramafic lavas rescaled to ignore the water content of the rock. The TiO_2 against MgO graph illustrates the method of estimating mantle composition. Regression lines with MgO as the independent variable give liquid compositions at 22% and 32% MgO (Table 1). X is the composition of the solid residue added to the 22% MgO liquid to give the 32% MgO liquid during partial melting

(assuming that TiO_2 is partitioned completely into the liquid). The solid residuum in equilibrium with the 32% MgO liquid is constrained to lie between X and an olivine composition of $\text{Fo}_{93.5}$ (see text). π = Archaean pyrolite. The shaded area shows the range of possible source compositions if the residuum evolves towards olivine.

unconformably on granitic crust⁵. We assume that the occurrence of pillow lavas in this sequence containing both skeletal olivine spinifex ($\text{Fo}_{92.5}$ in a rock with 28% MgO , E. G. Nisbet, M. J. Bickle and A. Martin, unpublished) and skeletal olivine microphenocrysts indicates that these particular lavas have a composition close to that of the liquid.

Here we present an analysis of these rocks. Only analyses of pillow lavas and thin (2–12 m) flows are considered since rocks with coarse spinifex zones or thicker ultramafic layers are less likely to represent liquid compositions. Because all of these lavas have undergone low greenschist metamorphism (relict clinopyroxene and olivine are preserved)⁶ we include only

Table 1 Compositions of various rocks

	Liquid composition at 22% MgO (calculated from Fig. 1)	Liquid composition at 32% MgO	Composition of X	Composition of Fo _{93.6} olivine in a 33% MgO lava ⁷	Possible source composition if 32% MgO liquid (2) is in equilibrium with a residuum of olivine and orthopyroxene in same ratio as X (olivine Fo ₉₁ , orthopyroxene En _{92.5})	Possible source composition if 32% MgO liquid is in equilibrium with Fo _{93.6} olivine	Average garnet lherzolite xenoliths in kimberlite ¹
SiO ₂ (%)	49.0	46.9	44.5	41.41	45.9	44.8	46.2
Al ₂ O ₃	7.7	5.3	2.7	0.0	2.2	2.7	2.16
Fe ₂ O ₃	1.3	1.1	1.0	0.0	0.4	0.6	—
FeO	10.2	9.2	8.1	6.27	8.6	8.1	6.82
MgO	22.0	32.0	43.2	51.6	40.7	40.8	42.0
CaO	8.6	4.5	0.0	0.19	1.7	2.4	1.64
MnO	0.19	0.16	0.11	0.12	0.08	0.11	0.11
Na ₂ O*	—	—	—	0.0	0.1	0.1	0.17
K ₂ O*	—	—	—	0.0	0.025	0.025	0.15
P ₂ O ₅ *	—	—	—	0.0	0.001	0.001	—
NiO	0.12	0.21	0.32	0.51	0.16	0.20	0.31
Cr ₂ O ₃	0.4	0.43	0.48	0.18	0.20	0.24	0.35
TiO ₂	0.4	0.22	0.0	0.0	0.08	0.11	0.12
Ce (p.p.m.)†	2.4	1.4	0.011	0.014	0.45	0.78	—
Yb†	0.91	0.60	0.017	0.006	0.20	0.33	—

*Values for Na₂O, K₂O and P₂O₅ in columns (5) and (6) are approximated using analyses from thicker but less altered ultramafic units containing euhedral olivines (E. G. Nisbet, M. J. Bickle, and A. Martin, unpublished data).

†Olivine:liquid and orthopyroxene:liquid distribution coefficients of 0.01 and 0.002 for Ce and 0.01 and 0.1 for Yb (ref. 10), are used to calculate their concentrations in columns (3) and (4).

those elements which are relatively unaffected by low grade metamorphism. REE patterns are considered not to have been significantly altered during metamorphism if they are smooth (without any marked kinks or unevenness) and parallel in different samples of similar rock types.

The major and trace element composition of ten ultramafic lavas are plotted against their MgO contents in Fig. 1. Like some other Archaean ultramafic lavas, these have low incompatible element contents and CaO–Al₂O₃ ratios around unity. With the exception of Na₂O and K₂O all the variation diagrams show reasonable linear correlations (Fig. 1). The correlations permit an estimate to be made of the bulk composition of the material added to the melt (in a partial melting model) or removed from the melt (crystallisation model) as it progresses from 32 to 22% MgO. It is important to note that this material (henceforth termed X) need never have existed as a real solid. Its MgO content is taken to be that at TiO₂ = 0.0, since only olivine and orthopyroxene are likely to be in equilibrium with such magnesian liquids. The normative composition of X does in fact contain olivine and a significant amount of orthopyroxene. A similar MgO content is obtained for CaO = 0.0. Titanium was chosen as an incompatible element likely to be almost exclusively partitioned into basic melts⁶ (minerals from harzburgite nodules have a very low TiO₂ content).

The chemical variation in Fig. 1 suggests that the melts must have evolved leaving a residuum containing both olivine and orthopyroxene. Moreover, the graphs of Cr₂O₃ and Al₂O₃ against MgO indicate that reasonable initial mantle compositions of these phases cannot control these trends. We consider, therefore, that the melts evolved in equilibrium with a solid residuum containing high NiO olivine and high Cr₂O₃, Al₂O₃ orthopyroxene.

Some constraints may be placed on the likely composition of the source by attempting to sum the compositions of the melt and residual phases in their correct proportions.

Two estimates must be made: first, the MgO content of the source, from which the degree of partial melt needed to produce the 32% MgO liquid can be calculated. We arbitrarily chose MgO 41% (based on published analyses of ultramafic nodules). Second, we must estimate the composition of the residuum in equilibrium with the most magnesian liquid observed at the

surface (32% MgO). The Mg–Fe ratio of the residuum is likely to be greater than that of X.

Estimates of the source composition have been made for two cases; an olivine : orthopyroxene residuum in the same ratio as the normative content of X; and a pure olivine residuum (similar to the Fo_{93.6} olivine observed in an ultramafic lava⁷). Demonstrably neither of these situations can be correct but the actual source composition probably lay within these limits.

The computed major element contents are remarkably similar to that of garnet lherzolite nodules in kimberlites (Table 1). The total Fe and Al₂O₃ are slightly higher (Fe is dependent on the Mg:Fe ratio assumed for the residuum) and SiO₂ possibly lower than the average of lherzolite xenoliths (Table 1). If a mantle MgO content of 37% were assumed, the SiO₂ content would be higher and the TiO₂ and CaO content lower than pyrolite².

The concentration of the incompatible elements (TiO₂, CaO, K₂O, NaO, REE and to a lesser extent Al₂O₃ and MnO) in the source depend only slightly on the concentration of these elements assumed for the residuum (see Table 1). The calculated concentrations of compatible trace elements (NiO, Cr₂O₃) is, in contrast, strongly dependent on the composition assumed for the residuum.

The REE patterns of the komatiites exhibit a depletion in light (relative to heavy) rare earths (Fig. 2). The Ce and Yb contents of the source are reasonably well constrained between 0.51–0.78 and 0.23–0.33 p.p.m. respectively. These estimates are less than the average chondrite value for Ce and between 1 and 1.5 times chondrite for Yb and are significantly lower than previous estimates of REE abundances in the mantle (3–5 times chondrite, see ref. 8). This particular mantle segment was probably less depleted in light REE than the source of some modern mid-ocean ridge tholeiites⁹.

Our work shows that a source mantle similar in composition to modern garnet lherzolite nodules in kimberlites could have produced komatiitic liquids. A pyrolitic source, even when extrapolated to similar MgO contents, is enriched in TiO₂ and CaO and depleted in SiO₂ relative to the source of these komatiites. Finally, if the approximately chondritic value of REE abundance in the komatiite source is typical of large portions of the upper mantle, then the degree of total REE enrichment

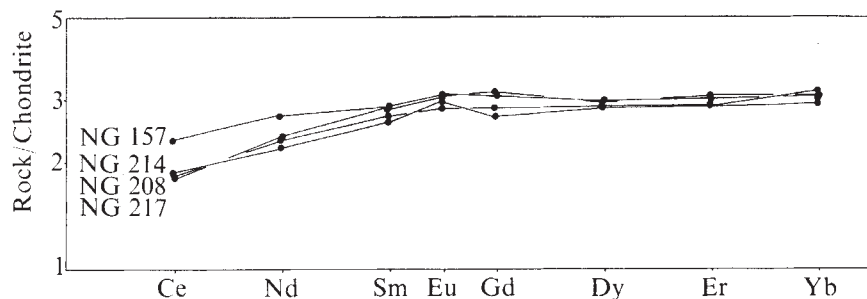


Fig. 2 Chondrite normalised rare earth distribution patterns from ultramafic lavas (23–30% MgO) Belingwe, Rhodesia. Analysis carried out by the mass spectrometric isotope dilution technique described by Hooker *et al.*¹¹.

(relative to source) observed in both Archaean tholeiites and mid ocean ridge basalts may be much greater than had previously been imagined^{8,12}.

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Photochemical ozone in the atmosphere of Greater London

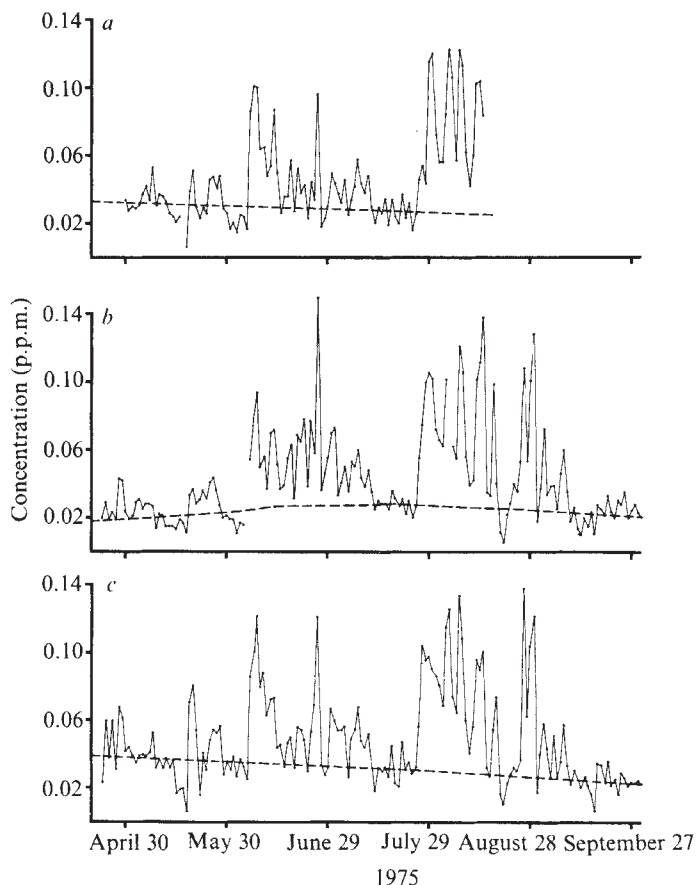
ATMOSPHERIC pollution in Greater London is usually associated with high concentrations of smoke and sulphur dioxide, but continual changes in human activity can lead to other types of pollution. One example is the reported occurrence of elevated ozone concentrations indicative of photochemical activity¹. I present details of the spatial and temporal variation of ozone concentrations experienced in Greater London during the summer of 1975. The data are presented, in part, with reference to the Greater London Council (GLC) guideline concentration for ozone of 0.08 p.p.m. (1-h average)^{2,3}. Observations indicate that photochemical ozone in and around London is likely to have a dual origin, namely, the European continent (see ref. 4), and photochemical reactions involving locally emitted precursor pollutants.

During the period May–September 1975 the Scientific Branch of the GLC monitored ozone at three sites in Greater London, two of which were located in suburban areas at Teddington and Hainault, with the third at County Hall in the centre. Teddington lies 18 km south-west and

Hainault 19 km north-east of the centre. The suburban sites were equipped with ozone detectors operating on the principle of ultraviolet absorption. At County Hall a chemiluminescent ozone analyser was used. All three sites were as far removed as possible from local sources of pollution, especially heavily-trafficked roads. The ozone instruments were cross checked and calibrated at the beginning and end of the season against a constant ozone source, itself standardised against a neutral-buffered KI solution⁵. Further cross checks were made between the two types of instrument in polluted atmospheres. Ozone concentrations were recorded at 30-s intervals on magnetic tape suitable for computer processing.

Figure 1 shows maximum hourly mean concentrations of ozone at each site during the monitoring period. Occurrences of elevated ozone concentrations are superimposed on a background concentration of 0.02–0.04 p.p.m. The background seems to represent the natural ozone content of the troposphere in this region, and is consistent with measurements reported for other parts of Britain⁴. With reference to the GLC guideline concentration the first conclusions that can be drawn are:

Fig. 1 Daily maximum hourly means of ozone: a, Hainault; b, County Hall; c, Teddington.



- During May to September 1975 ozone concentrations exceeded the GLC guideline on approximately 15% of days.
- The guideline was exceeded for approximately 100 h at Hainault, 79 h at County Hall and 129 h at Teddington during this period.
- The highest hourly mean concentration recorded at these sites was 0.15 p.p.m. at County Hall, on June 26, 1975.

A study of the meteorological records of the London Weather Centre shows that days with high afternoon ozone concentrations generally coincide with both low early morning wind speeds, usually of less than 3 m s^{-1} , and high solar radiation⁶. An association of oxidant incidents with inversion conditions as measured at Cardington, Bedfordshire, has also been found; it is statistically significant at the 0.1% level. During the summer of 1975 the GLC guideline was exceeded at one or more of the Greater London sites on 56% of occasions on which the 0500 Balthum ascent from Cardington registered an inversion strength of 4°C or more. Conversely, for weaker or nonexistent inversions, the guideline was exceeded on only 4% of occasions. Figure 2 shows such information for the period June 25–27, 1975. The key factor in the ozone incident of June 26 is thought to be the development of a strong inversion during the previous night, which encouraged the buildup of locally generated oxidant precursor pollutants, oxides of nitrogen and hydrocarbons. These subsequently became involved in photochemical reactions during the sunlight hours of June 26. The sequence was not repeated the following day, when overnight cloud cover inhibited inversion formation.

Isolated occurrences of high ozone concentrations, as on June 26 were, however, uncommon. Generally, days with high ozone concentrations tend not to occur singly, but over periods of several days or more⁷, and Greater London was no exception during 1975, with the main oxidant incidents

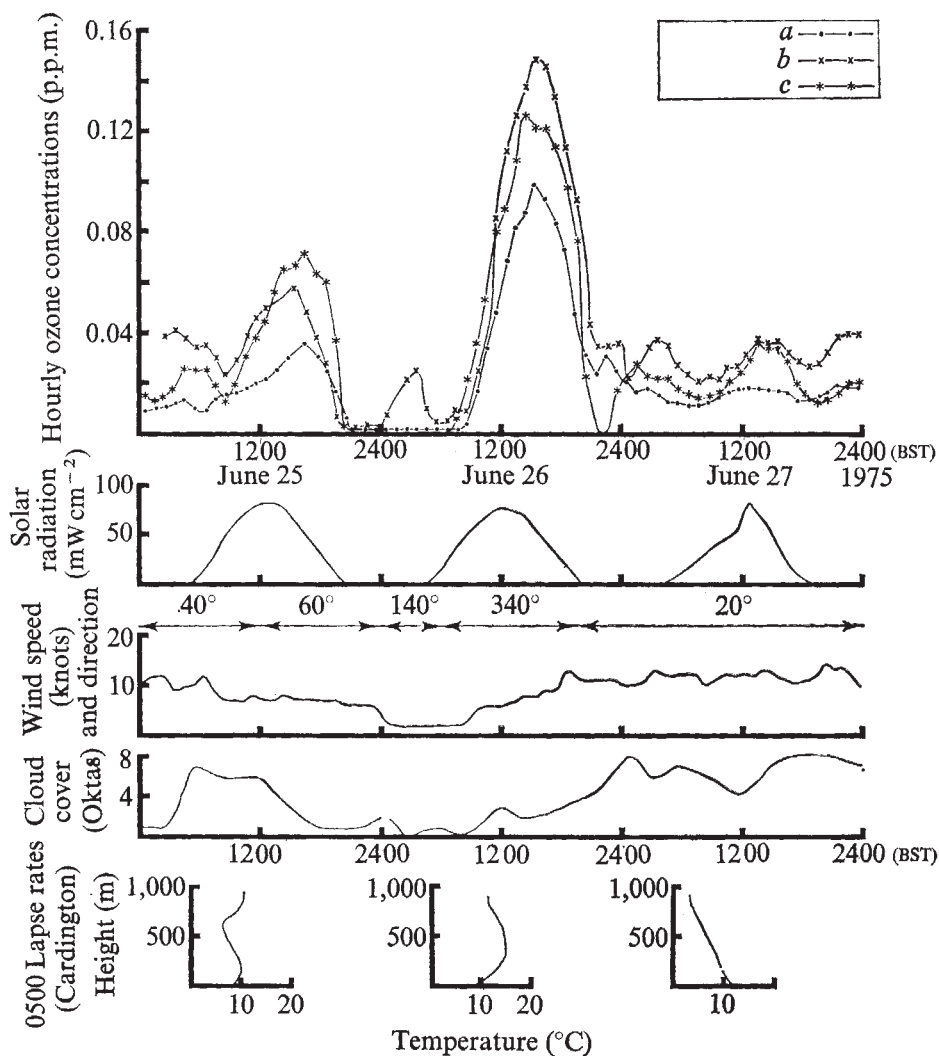
Table 1 Comparison of wind direction (1100–1500) and ozone concentrations at Teddington and Hainault

Date (1976)	Maximum hourly mean	Ozone concentration (p.p.m.)	Wind direction (degrees)
	Teddington	Hainault	
June 8	0.122	0.100	060
June 10	0.088	0.065	020
June 13	0.073	0.087	290
July 27	0.104	0.054	120
July 31	0.087	0.072	080
August 1	0.081	0.056	060
August 3	0.116	0.084	120
August 13	0.090	0.103	190

occurring during June 7–12 and July 27–August 14. During the latter period the guideline was exceeded on 14 of the 19 days.

A study of the appropriate Daily Weather Reports shows that on all days with high oxidant levels the synoptic conditions were dominated by slow moving anticyclonic cells

Fig. 2 Ozone and meteorological data for the period June 25–27, 1975: *a*, Hainault; *b*, County Hall; *c*, Teddington.



and/or weak synoptic pressure gradients. Anticyclonic conditions are generally characterised by light winds or calm conditions with clear skies. The latter lead to nocturnal inversion, which in turn encourages the buildup of precursor pollutants throughout the whole anticyclonic cell, and the consequent production of oxidants over large areas at any one time. Because the anticyclones are slow moving the conditions tend to persist for days at a time. It is also apparent that on most occasions these cells were centred on the North Sea, North Germany, or Scandinavia. Thus, southern Britain, including the Greater London area, would have been experiencing a light and predominantly easterly air stream of continental origin. This accords with the observations of Cox *et al.*⁴, which indicate that photochemical ozone and its precursors may be transported into Britain from continental Europe. In fact, examination of air mass trajectories for 1975 by using the geostrophic wind, as calculated from the Daily Weather Report, shows that most air masses over Greater London on high oxidant days were of continental origin⁵.

But a close inspection of the relative values of the maximum hourly means at the Teddington and Hainault sites on high oxidant days on which there was a significant difference in concentration between the two locations shows that the higher concentration is manifest at the site for which the incoming air mass had had the longest trajectory over Greater London (Table 1). In addition, on days characterised by exceptionally long periods with low wind speeds, such as June 26, the highest measured concentration in London, and indeed in the whole of Britain (H.N.M. Stewart, personal communication), occurred in the city centre.

The most likely explanation for these phenomena is that emissions of precursor pollutants from Greater London are reacting photochemically to produce oxidants. The locally generated precursors drift downwind during the late morning and early afternoon and produce higher ozone concentrations on the downwind side, and possibly higher concentrations still beyond the GLC boundary. On particularly calm days the precursors do not drift as far and maximum ozone concentrations tend to arise close to the maximum density of emissions, which is probably in central London. This ties in with the earlier mentioned observation that the GLC guideline was exceeded more in west than in east London, because local winds have tended to be from the eastern sector on high oxidant days.

The picture which emerges is of two sources of oxidants contributing significantly to high ozone concentrations in the atmosphere of Greater London. First, during anticyclonic weather in northern Europe precursor pollutants collect over large areas in what virtually amounts to an outdoor smog chamber. Widespread elevated ozone concentrations are thus formed more or less simultaneously. Ozone, which is rapidly destroyed at the Earth's surface, is then able to persist for several days because low level nocturnal inversions, which form frequently under anticyclonic conditions, inhibit downwind mixing. This allows its transportation over distances of perhaps 1,000 km or more⁴, with a possible influx to Greater London. Second, the same synoptic weather conditions favour the simultaneous buildup of locally generated precursor pollutants, and the subsequent formation of oxidants in London's atmosphere.

With forecasts that road traffic will increase significantly in the next decade, continued vigilance is necessary so as to detect, at an early stage, any deterioration in the situation. Although it is apparent that corrective action, should it be required, must be taken in the context of Europe as a whole, the problem of local generation of oxidants warrants individual study as the optimum abatement strategy may vary from one region to another.

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Ozone levels in central London

MEASUREMENTS of ozone and some primary pollutants, including oxides of nitrogen and total hydrocarbons, have been in progress at the Central London background site of this laboratory since July 1972. A preliminary note¹ discussed the early results from this site; here we present a statistical analysis of the relationship between ozone levels in Central London and some meteorological parameters measured at the London Weather Centre, ~0.4 km from the sampling site. The data were collected during the periods July-September 1972 and April-September in both 1973 and 1974.

The sampling point at the site, which is located at Endell Street, Holborn, London, is ~10 m above ground, well removed from traffic or any particular pollutant source and is not unrepresentative of the background air in Central London.

An analysis of the time of occurrence of the ozone maximum hourly mean concentration for all the days considered is summarised in Fig. 1. This conforms to the general pattern found in other studies^{1,2} with a small percentage of maxima occurring in the earlier hours of the day, arising from the diffusion of stratospheric ozone and the majority of maxima, from photochemical activity, occurring between 1500 and 1600 LT. It is interesting to note that relatively high (>10.9 parts per 10⁸) levels were observed late in the day (1900-2100 LT) on some occasions, a fact which has been taken by Steinberger and Balmor³ to be indicative of non-local production of ozone.

Fig. 1 Time of occurrence of the maximum hourly mean ozone concentration at Endell St, London for the summer periods 1972-1974.

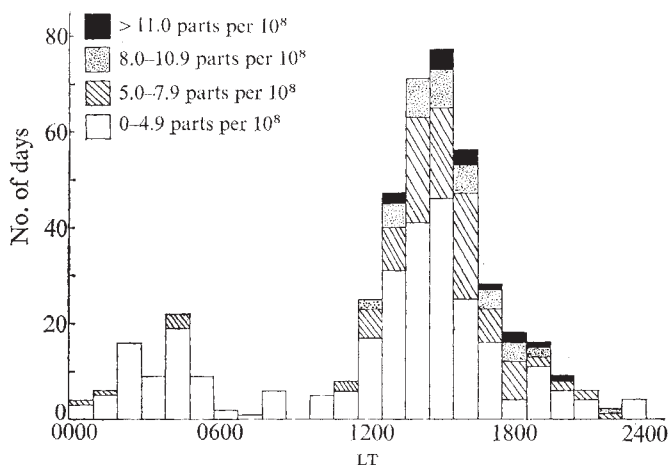


Table 1 Analysis of the dependence of elevated ozone levels on wind direction

	N*	Wind direction						
		NE	E	SE	S	SW	W	NW
No. of days in given direction	51	67	51	21	40	80	72	38
% of total	12	16	12	5	10	19	17	9
No. of days with $[O_3] \geq 8.0$ p.p. 10^8 in given direction	4	13	11	5	9	6	6	3
No. of days with $[O_3] \geq 8.0$ p.p. 10^8 as % of total in given direction (f)	8	19	22	24	23	8	8	8

*Any direction from 337.5° – 22.5° is taken as N, 22.5° – 67.5° as NE and so on.

An attempt was made to correlate the daily ozone maximum hourly mean concentration with average values for the whole day and the period 0600–0900 h for the primary pollutants NO , NO_x and total hydrocarbons. No significant relationship was found.

A preliminary bivariate analysis of the ozone levels and selected meteorological parameters indicated that the most important factors were maximum daily temperature, relative humidity and daily insolation. A stepwise multiple regression analysis was then performed using the logarithms of these

correlation coefficient improved to 0.712. The regression equation obtained was, in an obvious notation

$$[O_3]_t = 0.68 [O_3]_{t-1}^{0.38} T_t^{0.37} I_t^{0.28} H_t^{-0.36} \quad (2)$$

The partial correlation coefficients of the independent variables are, in the order in which they appear in equation (2), 0.44, 0.19, 0.24 and -0.17 , (all significant at the 1% level).

The standard error in reproducing observed ozone levels using this model is ± 2.1 parts per 10^8 (see Fig. 2) so that in predicting elevated levels, that is, those ≥ 8.0 parts per 10^8 this implies a confidence limit of $\pm \sim 26\%$. We therefore present equation (2) as a model for ozone concentrations at the Central London background site.

We have attempted to analyse the effect of wind direction as measured at the London Weather Centre on the ozone levels. The results of this analysis are shown in Table 1 and a plot of the percentage of days with wind from a given direction when levels ≥ 8.0 parts per 10^8 occurred is shown in Fig. 3. It is clear from this histogram that the majority of elevated zone levels occur on days when the wind is from a direction ranging from north-east through to south. These conditions prevail, in general, when an anticyclone is present over the North Sea or north-western Europe when the air flow is from the Continent.

It should be borne in mind that the wind direction as measured at a particular location, in our case the London Weather Centre,

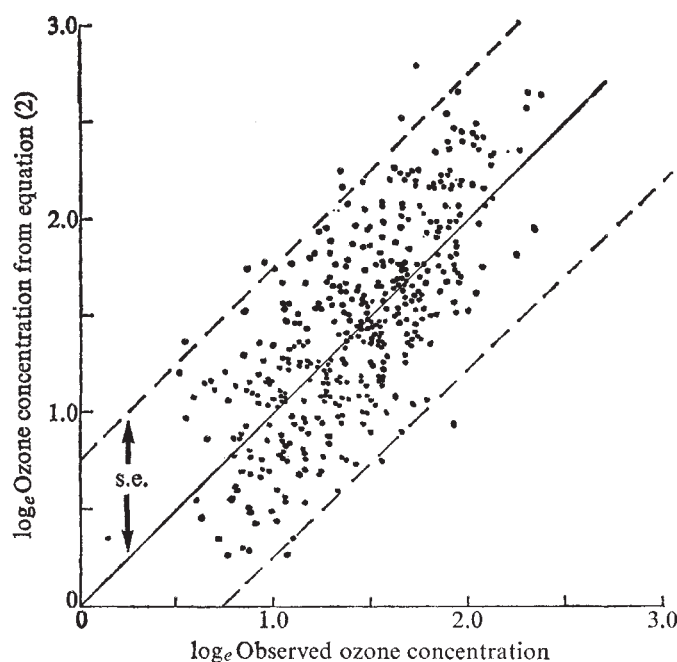


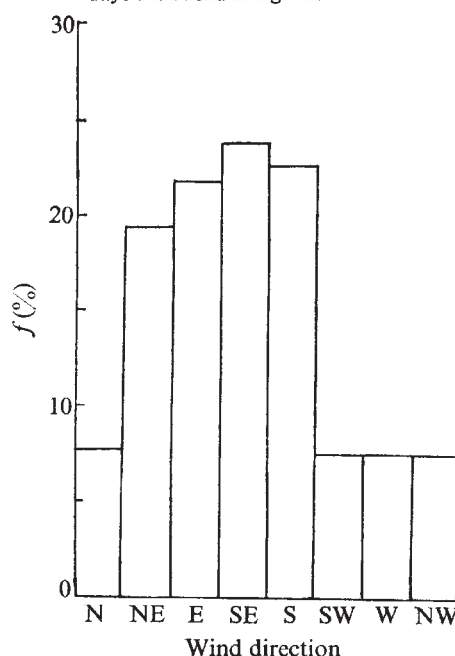
Fig. 2 Plot of values of ozone concentrations calculated from equation (2) against observed values.

parameters as independent variables with the logarithm of the ozone concentration as dependent variable. The number of days for which valid data were available was 453. The regression equation obtained was

$$[O_3] = 0.60 T^{0.64} I^{0.31} H^{-0.43} \quad (1)$$

where T is the maximum daily temperature ($^\circ C$), I is the total daily insolation ($mWh\ cm^{-2}$) and H is the relative humidity (%). The multiple correlation coefficient obtained was 0.623. Adding wind speed to the regression gave no significant improvement. An analysis of the residuals yielded a Durbin-Watson statistic of 1.145 indicating autocorrelation in the ozone levels. On including the logarithm of the previous day's ozone concentration in the set of independent variables the multiple

Fig. 3 Plot of number of days with ozone concentration ≥ 8.0 parts per 10^8 expressed as a percentage (f) of the total number of days with wind in a given direction.



is not necessarily a precise indicator of the actual trajectory of the air mass which might be responsible for the transport of pollutants. Consequently further refinement of the model to include wind direction in a more quantitative manner, by stratifying the data in wind sectors for example, was not felt to be justified.

From a consideration of the wind direction analysis and the times of occurrence of the elevated ozone levels it is likely that a significant proportion of the ozone measured at Endell Street is transported there, but further investigation would be necessary to determine the source areas. One possibility is that the continental air mass contains precursor pollutants and produces ozone as it flows over north-western Europe giving rise to elevated levels even at rural sites well away from sources of pollution. Elevated ozone levels measured at Adrigole on the West Coast of Ireland lend support to this idea and Cox *et al.*² have discussed this point at length, concluding that ozone and precursors could be transported over distances up to 100–1,000 km.

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Gravity counteracts light-induced inhibition of root growth

THE view that roots are generally indifferent to light has recently been revised in the face of evidence that light can be inhibitory to the growth of some roots^{1–10}. The discovery^{6,11} that light affects the production of an inhibitor prompted the suggestion⁸ that sensitivity to light in roots is related to the system controlling geotropic response, which is also dependent on the presence of an inhibitor^{12,13}. Since the onset of geotropic responsiveness in some roots is dependent on exposure to light^{14–16}, the suggestion seems well founded and attempts are being made to clarify the extent to which the mechanisms, triggered when light or gravity affects the root, are interrelated. The investigation described here was prompted by the report⁹ that roots of cress seedlings grown in Petri dishes in the light were stunted in comparison with similar seedlings in the dark. If light promotes geosensitivity, distorted growth could develop in a situation where the root is unable to realign itself, as for example when it is restricted to the horizontal plane. A study was therefore made of the influence of the plane of growth on the light-induced inhibition of root

elongation in cress seedlings, from which it seems that although light does not enhance geosensitivity, light-induced inhibition is manifest only in horizontally grown roots and can be prevented by subjecting the roots to a force field.

Seeds of *Lepidium sativum* L. cv. Curled were moistened with 0.1 mM CaCl₂, pH 6.5, and left for 20 h at room temperature in normal lighting conditions. Seeds which were at a uniform stage of germination—the testa having split open but before the emergence of the radicle—were then placed with the radicles pointing downwards on a 11.5-cm circle of Whatman No. 1 filter paper in a glass Petri dish (115×18 mm) containing 6 ml of 0.1 mM CaCl₂. For dark treatments Petri dishes were either sprayed externally with black paint or wrapped in aluminium foil. The dishes were placed on a bench continuously illuminated by a bank of fluorescent tubes giving an incident light intensity of about 20 W m⁻² at the level of the Petri dishes. The dishes were placed horizontally, parallel to the light source, or at angles of 10°, 45° and near vertical (85° to 90°). For the angled dishes side illumination was also provided but growth showed no dependence on the direction of the light source. Cress roots are not negatively phototropic. Root lengths were measured at 24-h intervals after treatment began, that is approximately 44, 68 and 92 h after the seed was wetted.

Table 1 shows a comparison of root growth of seedlings in the light and in the dark as affected by the plane of growth. The reported inhibition⁹ of root growth in the light was evident in horizontal roots at 44 h, and this inhibition continued into the following 2 d, although with time the growth rate of horizontal roots in the light increased. With vertically orientated seedlings in the light there was no inhibition of root growth relative to horizontal roots in the dark. The ability of light-exposed roots to overcome light inhibition is clearly a function of the plane of growth, an angle of 10° having a discernible effect. At 45° the growth rate was not far short of that obtained on the vertical. Since the component of the Earth's gravitational field in the plane of growth is determined by the sine of the angle subtended with the horizontal, an angle of 45° would produce a force of approximately 0.7g and therefore it would be expected that this slope would give a growth rate approaching that of the vertical. Roots growing in the dark also showed a positive response to being aligned with the Earth's gravitational field but the percentage increase was much less than in the light.

These results indicate that when the force of gravity acts on a seedling growing in a vertical plane the light-induced retardation of growth observed in the horizontal plane is largely eliminated. Two further questions are thus raised. First, is horizontal root growth inhibited in the light in the absence of gravity and second, is horizontal root growth inhibited in the light when the root is subjected to a force field applied on the horizontal plane? An answer to the first question was sought by placing imbibed seed on moist filter paper on the base of a crystallising dish which was illuminated and, with the base vertical, was then rotated

Table 1 Effect of the plane of growth on the length (mm) of cress seedling roots in the light and dark

Treatment	Time of measurement (h)	Angle subtended with the horizontal			
		0°	10°	45°	85°
Light	44	6.1±0.28	10.5±0.39	12.8±0.40	16.1±0.49
	68	15.7±0.66	35.3±1.73	43.2±0.90	50.5±1.06
	92	30.5±0.91	69.4±2.82	76.8±1.62	90.7±1.86
Dark	44	14.7±0.55	17.1±0.63	20.3±0.51	21.3±0.46
	68	39.5±1.42	51.5±1.31	56.0±1.35	60.2±0.71
	92	65.6±2.44	79.7±2.24	80.3±1.64	88.6±1.99

Twenty seeds were placed in Petri dishes as described in the text and the growth of the roots was measured over 4 d at 25 °C. The Petri dishes were clamped at the angles shown and care taken to ensure uniform illumination at an intensity of approximately 20 W m⁻². Each treatment was in duplicate and the values shown are the mean ± s.e. of 40 seedlings.

round a horizontal axis in the manner of a clinostat. The second question was answered by placing a circle of imbibed seed so as to form a hub on filter paper in a Petri dish which was then illuminated and spun at 150–200 r.p.m. (1–2g) in a horizontal plane. Table 2 shows that where the effect of gravity was neutralised, and with the root free from the restraints imposed by a horizontal barrier, a light-induced inhibition of root growth was still evident. But this inhibition could be overcome on the horizontal plane by applying a centrifugal force comparable with that of gravity.

The question posed above concerning the geosensitivity of cress roots in light and dark was answered by making a 90° rotation of Petri dishes containing seedlings growing vertically in the light and in the dark. In both cases the roots reorientated themselves with the field of gravity irrespective of whether they were, or had been, in the light or in the dark. The light-induced root inhibition exhibited by horizontally grown seedlings is not therefore attributable to an enhanced geosensitivity not possessed by the seedlings grown in the dark.

Table 2 Effect of force field on the growth of cress seedling roots

Treatment	Radicle length (mm)
Light horizontal	6.7±0.38
As above with centrifugal force	17.8±0.71
Gravity neutralised	
dark seedlings	14.0±0.71
light seedlings	6.2±0.20

Seeds were imbibed for 20 h and then 20 seeds were placed in each of the dishes as described in the text and subjected to treatment for the following 24 h at 25 °C. The centrifugal force applied on the horizontal plane was between 1 and 2g. Gravity was neutralised by rotating the vertically-orientated dishes round a horizontal axis at 5 r.p.h. Values shown are mean ± s.e. for each treatment.

It is evident that in cress roots, growth is promoted by the longitudinal force exerted by gravity, one effect of which is to counteract the inhibitory effect of light. Not only root growth is promoted. As the steepness of the plane of growth increases so does the growth of the seedling, the cotyledonous leaves emerging and greening more rapidly than when horizontal. This points to an overall effect of gravity on the rate of hormone production or transport or both.

The cress root is a classic object for the study of amyloplasts, the gravity-sensing organelles present in the root cap^{17,18}. Audus¹⁹ has suggested, on the basis of work by Sievers and Volkmann²⁰, that mechanical pressure exerted by the amyloplasts on the membranes of the endoplasmic reticulum, or some other non-migratory cellular component, could result in the production of a regulatory compound in the root cap. This is envisaged in terms of a differential flow of inhibitor leading to a curvature of the root, but it seems reasonable that a preferred orientation of amyloplasts should also give a minimum flow of inhibitor or an optimum flow of regulators.

It has been suggested that root growth is controlled by two hormonal systems^{19,21}. Light is known to promote the acropetal movement of indolyl acetic acid in *Zea* roots¹⁵ and it has been reported that the gravity-promoted basipetal movement of a growth inhibitor in *Zea* is dependent on light^{8,22}. The results reported here show that gravity can interact with light antagonistically in the overall control of root growth. Identical results have also been obtained with seedlings of *Lactuca sativa* and *Brassica hirta* in both of which the roots, in contrast to those of cress, are strongly negatively phototropic, and in both of which a pronounced light-inhibition on the horizontal plane is relieved on the vertical plane. This phenomenon might prove useful in the investigation of the changes in hormone production and

transport brought about by amyloplasts sedimenting under the influence of gravity.

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Heterochromatin polymorphism and colour pattern in the tiger swallowtail butterfly *Papilio glaucus* L.

THE difference between the black mimetic and yellow non-mimetic female forms of the tiger swallowtail butterfly, *Papilio glaucus* L., is probably controlled by a locus on the Y (W) chromosome (cytoplasmic inheritance is less likely). Since the female is the heterogametic sex in the Lepidoptera black females should produce only black daughters and yellow females only yellow ones, and here we report cytological differences between the two.

It is known that occasional yellow females arise from black ones and vice versa, and in our broods four black females gave rise to a minority of yellow daughters, and two yellow ones to a minority of black¹. Haldane² suggested that these exceptional broods might be explained by occasional crossing over between the X and the Y chromosome, plus a position effect, such that black pigment is only laid down by the appropriate allelomorph when this is on the Y chromosome. Furthermore, he thought a position effect to be particularly likely because of the tendency for inert chromatin to be present on the Y chromosome.

The difficulty with Haldane's explanation of the exceptional *glaucus* broods is the fact that crossing over is believed not to occur in female Lepidoptera^{3–5}. Our recent cytological evidence however, lends striking support to Haldane's view that heterochromatin is involved in the expression of the black phenotype.

We have examined some of our *P. glaucus* males and black and yellow females and found that the black females possess and the yellow ones lack the heteropyknotic sex chromatin body (Fig. 1) described by Smith⁶ and later shown to be the heterochromatin on the Y (W) chromosome⁷. The yellow butterflies tested were the male and female descendants of two wild yellow females from New Jersey, and the female descendants of a yellow female from West Virginia. All were Smith negative. Among the descendants of three wild black females from West Virginia and one from Chicago, the males were again negative, but in contrast all the females were Smith positive. Thus the black females and the (yellow) males are characteristic of the cytological picture of most Lepidoptera, whereas the yellow females are behaving like those of a minority of species where no heterochromatic body is found in either sex⁸. In the only other minority category known to occur, there is a body in both sexes, and in at least one species (*Papilio machaon* L.) the male is polymorphic for the presence or absence of the body (our work with W. Traut, in preparation).

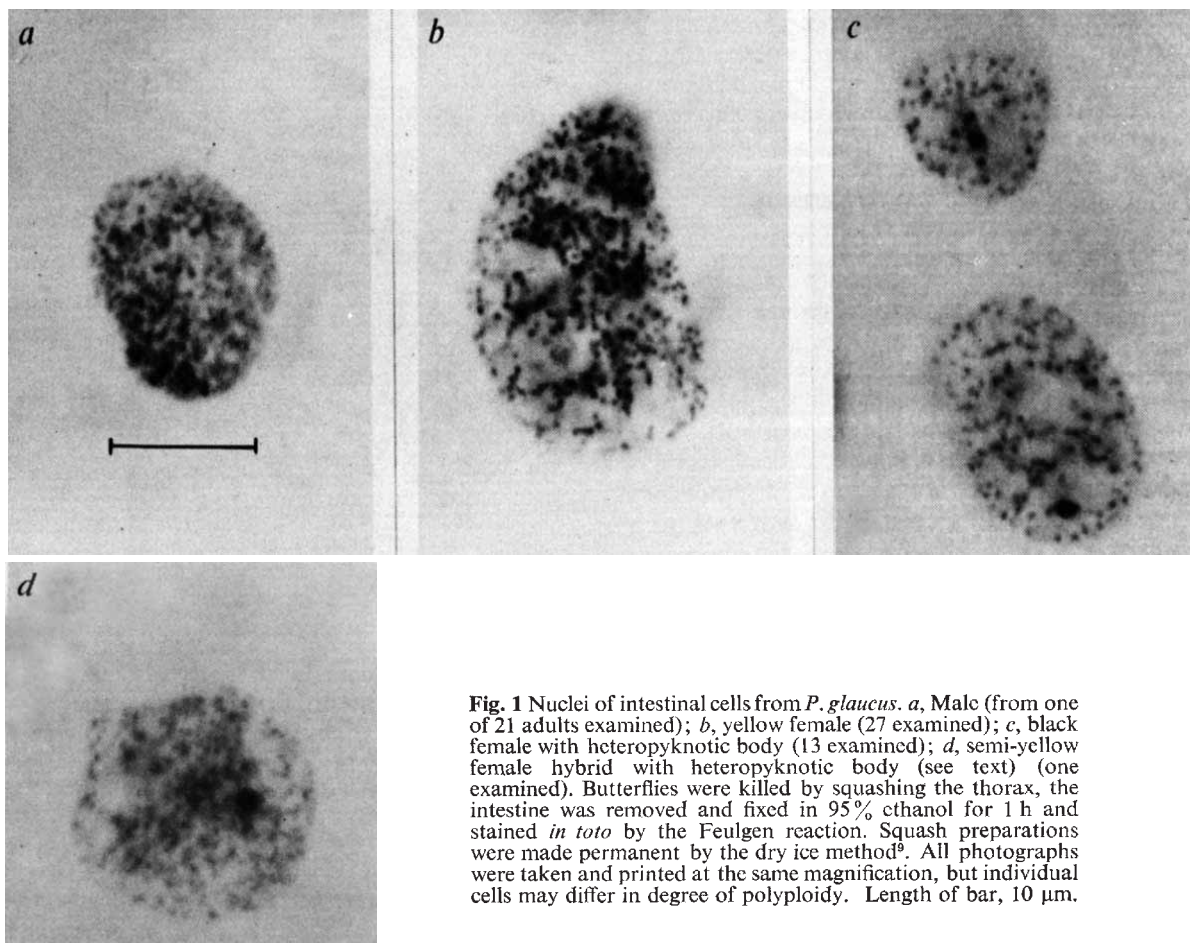


Fig. 1 Nuclei of intestinal cells from *P. glaucus*. *a*, Male (from one of 21 adults examined); *b*, yellow female (27 examined); *c*, black female with heteropyknotic body (13 examined); *d*, semi-yellow female hybrid with heteropyknotic body (see text) (one examined). Butterflies were killed by squashing the thorax, the intestine was removed and fixed in 95% ethanol for 1 h and stained *in toto* by the Feulgen reaction. Squash preparations were made permanent by the dry ice method⁹. All photographs were taken and printed at the same magnification, but individual cells may differ in degree of polyploidy. Length of bar, 10 μ m.

P. glaucus is widely distributed in North America, and we have not examined sufficient females from different localities to be certain that the 1:1 correspondence between female colour and heteropyknotic state in our study is not due to geographical heterogeneity in the cytological picture. The correspondence, however, suggests that in the exceptional segregating broods where the mother is black, the Y chromosome bearing the locus for the black gene is occasionally lost from the eggs of black females, who would then produce yellow daughters. It is more difficult to see how the body could be gained as it would have to come from a non-homologous chromosome. Possibly relevant is the fact that, as we noted¹, all our exceptional broods stemmed from females which may well have been related and have a chromosome abnormality resulting in loss or gain of heterochromatin by the Y.

There is another aspect of the Smith body in our work on *P. glaucus*. To be certain that the exceptional females were not mistakes due to the interchange of larvae between broods, we hybridised black *P. glaucus* females with the yellow monomorphic allopatric species *P. rutulus* Lucas because the hybrid larvae are recognisable. This cross produced no adult females, so we backcrossed the male progeny to black *P. glaucus*. We obtained some broods which segregated for black and yellow females and others in which a proportion of the black females had various degrees of yellow scaling, but none was fully yellow. The segregation (25 pure black in 41 females) suggests a 1:1 ratio, a situation quite different from that in pure *P. glaucus*. Since we began to use the Smith technique we have found that the only hybrid semi-yellow female (dissected and found to be a normal female) that has been tested possesses the heterochromatic body characteristic of black *P. glaucus* (Fig. 1d): two of her all-black sibs also had the body. In the progeny of the backcross to yellow the body was absent.

The segregation of yellow and black in the *P. rutulus* hybrids seems therefore to be due to an allelomorph suppressing black, introduced from *P. rutulus*. This is supported by the fact that the

segregation in the backcross occurs in the progeny of females that were pure black *P. glaucus*. It seems therefore that the black form can be altered in two ways, either by bringing in a modifier not on the Y chromosome from the allied *P. rutulus*, or by a change on the Y chromosome associated with the absence of the heteropyknotic body within *P. glaucus*.

It is important to know the state of the heteropyknotic body in the three allopatric species *P. rutulus*, *P. multicaudatus* Kirby and *P. eurymedon* Lucas, since the ancestral cytological state is likely to have been that found in black *P. glaucus* today (the majority situation) and it is interesting from the evolutionary point of view to know whether the heteropyknotic body has been lost and regained, or merely lost in yellow *P. glaucus*.

We thank Mr George Barrett, Mrs Alison Gill and Mrs Winifred Cross for technical help, and the Nuffield Foundation, The Royal Society and the SRC for support.

Since our manuscript was submitted adult females have been obtained from black *P. glaucus* females and *P. rutulus* Lucas. On July 8, 1976 two perfect females were made to eclose by Dr Axel Willig of the University of Ulm, using α -ecdysterone. The insects resemble the yellower intermediate backcross females and have a Smith body.

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Diet influences attractiveness of urine in guinea pigs

INTRASPECIES chemical communication with urine-borne substances is widespread in mammals. Numerous reports have indicated that the nature of the messages transmitted in urine depends on such factors as the hormonal state, the age, the individual identity and the level of stress of the sender^{1,2}. I report here that information transmitted in urinary signals also depends on the diet of the sender. These findings have important implications for the study of animal chemical communication systems³.

Chemical signals have an important role in social interactions of the guinea pig. In seminatural settings, subordinate females spray males and other females with urine whereas males often spray females during courtship. Scent-marking behaviour in this species results in placing urine as well as glandular secretions throughout the environment^{4,5}. In laboratory studies, males spend more time investigating female conspecific urine than male conspecific urine, urine from other species, or water^{6,7}.

To examine whether the diet of an animal influences the information contained in the urine, 12 individually housed, sexually mature, socially experienced male guinea pigs were given a series of preference tests (single-sample tests and two-choice tests) between urine collected from donors on different diets. The tester was always unaware of the source of the urine being tested. A finding that urine from one source is preferred to urine from a second source is evidence that the two samples possess different information. The urine to be tested was collected from adult, intact sexually non-receptive female and adult male guinea pig donors using metabolism funnels. Different donors were used in each experiment. All urine was frozen until shortly before use. In the first experiment, urine from four male and four female donors was used. Twenty-two hours before the first urine collection two female donors and two male donors, randomly selected, were provided with crushed (Purina) rat food (RF) and water. The other donors remained on (Wayne) guinea pig food (GPF). All donors' food was removed and urine was collected during the next 2 h. Urine from donors eating the same food (GPF or RF) and from donors of the same sex (♀♀ or ♂♂) was pooled and two series of single sample tests^{6,7}, separated by 2-d rest, were conducted. Each male was tested with ♀♀ GPF urine, ♂♂ GPF urine, ♀♀ RF urine, ♂♂ RF urine and water. The weights of the donors either increased slightly or remained constant during the 22 h on experimental diets.

The results (Fig. 1) show that females on a RF diet produce urine which is less attractive than that of females eating GPF. This occurred even though the donors were fed the new diet for only 22 h preceding urine collection. Even though the attractiveness of ♀♀

RF urine is, however, depressed, this urine is still investigated more than ♂♂ GPF urine, confirming the previously documented preference for female urine over male urine by male recipients^{6,7}.

To examine the generality of this finding, urine from female and male guinea pigs fed a semisynthetic diet (Reed-Briggs) was compared with urine from the same animals fed GPF using a simultaneous two-choice method^{6,7}. The urine collected when the donors were on a semisynthetic food was less attractive than urine collected when they were on GPF (Table 1, experiments 1 and 2), in agreement with the RF results. When the donors were force fed a standard amount (approximately 16 g over 2 d) of either GPF or semisynthetic diet, the results were similar: GPF urine was

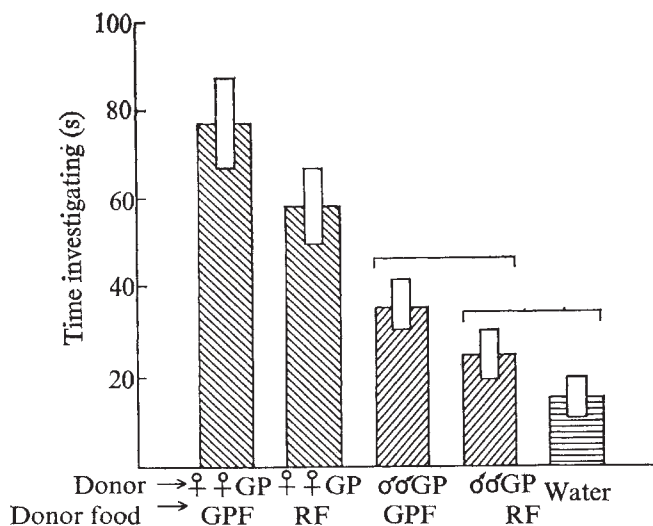


Fig. 1 Results of single-sample preference tests. The heights of the bars represent the mean ($\pm$ s.e.m.) time (s) that the male guinea pigs have their nose within approximately 1 cm. of the urine from four sources and water, each presented singly. The four sources are: ♀♀ GPF, urine that was collected from females eating guinea pig food during the 22 h preceding collection; ♀♀ RF, urine collected from females eating rat food over the same period; ♂♂ GPF, urine collected from males eating guinea pig food; ♂♂ RF, urine collected from males eating rat food; water, charcoal filtered and deionised water. For each substance, each animal had a total of two 2-min periods to investigate a sample (0.2 ml) which was placed in the centre of a clean glass plate (7.5 $\times$ 15.0 cm). Each sample was tested individually in the subjects' home cage, one sample each day. The order of presentation was randomised. Overall, there was a significant difference among the samples (represented measures ANOVA, $F(4,44) = 21.98$, $P < 0.001$). Those bars not connected by a horizontal line are significantly different from each other ($P \leq 0.05$, Newman-Keuls test).

Table 1 Mean time (s) investigating each of the two guinea pig urine samples in five preference experiments.

Experiment	Donors' Chow	Time (s)	Choice	Donors' Chow	Time (s)	P
1*	Guinea Pig	107.0 $\pm$ 18.7	v	Semisynthetic	44.1 $\pm$ 7.2	<0.01
2*	Guinea Pig	92.2 $\pm$ 14.1	v	Semisynthetic	36.3 $\pm$ 7.1	<0.01
3	Guinea Pig	95.0 $\pm$ 23.6	v	Semisynthetic	37.4 $\pm$ 8.7	<0.01
4	Guinea Pig	108.3 $\pm$ 21.2	v	Rat	50.8 $\pm$ 10.1	<0.01
5a	Guinea Pig	36.9 $\pm$ 4.7	v	Guinea Pig	30.8 $\pm$ 6.9	NS
b	Guinea Pig	57.2 $\pm$ 9.5	v	Rat	19.2 $\pm$ 4.8	<0.01
c	Guinea pig	33.2 $\pm$ 8.4	v	Guinea pig	33.4 $\pm$ 7.6	NS

Each experiment except 5 represents two 4-min choice tests with 12 or 13 subjects where the two samples to be compared were presented simultaneously to a subject; in experiment 5, only one 4-min choice test was conducted for each of the three tests. For all experiments the donors were four females except for choice 2, where four males served as donors. Probabilities based on two-tailed *t* tests for related measures. See text for further explanation.

*In choices 1 and 2, urine from donors fed GPF was diluted in the appropriate amount with deionised water before testing, since animals on semisynthetic diet drank more water and produced larger volumes of urine.

NS, not significant.

preferred to urine from donors force fed semisynthetic food (Table 1, experiment 3). One possible explanation for the pattern of results is that abrupt short term alteration of the diet stimulates physiological changes that result in the production and urinary excretion of some substance that the male guinea pig subject finds noxious. To examine this question, two female guinea pigs were raised from birth on crushed RF and water fortified with vitamin C. When the animals were well past sexual maturity, urine was collected and compared with urine from littermates reared on GPF from birth. A different group of 13 males was used as subjects in this test. As before, GPF urine was greatly preferred over RF urine (Table 1, experiment 4).

Different groups of animals served as donors for each experiment described above reducing the possibility that the differences observed could be accounted for by inherent differential attractiveness of urine from different animals. To eliminate completely this possibility, however, a final experiment was conducted. From four previously unused female donors of approximately equal age and weight, two were randomly selected to be on RF and the other two were to remain on GPF. Three pairs of urine samples were collected. Immediately before placing the donor pair on RF, urine was collected for 3 h from both pairs (a). Immediately after the one pair had eaten RF for 21 h, urine was similarly collected from both pairs (b). Finally, GPF was available to both pairs of donors then for the next 21 h after which urine was collected a third time (c). The amount of RF consumed during the 21 h (25 and 28 g for the two donors) was similar to the amount of GPF eaten in the same period by the two control donors (26 and 27 g); similarly, body weight remained constant. All females were sexually unreceptive during the 3-d period. In several previous studies we have failed to find any change in the attractiveness of female guinea pig urine as a function of the stage of oestrus. When the three pairs of urine samples were tested using the simultaneous two-choice procedure, it was found that urine from both pairs of donors which was collected before the diet was changed (experiment 5a) and urine which was collected after both pairs of donors had been returned to GPF for 21 h (experiment 5c) was equally attractive. Urine collected from donors immediately after one pair had been on RF for 21 h was, however, significantly less attractive than urine from donors on GPF (experiment 5b).

Leon⁸ has demonstrated that the food eaten by a mother rat influences the odours emanating from her caecal contents. His data further suggest that the pups were attracted to odours possessed by their mothers as a result of learning. Analogous hypotheses have been suggested by Hendry *et al.* for some insect pheromone systems³. In my experiments, the male guinea pig subjects experienced throughout their lives urine only from animals who had eaten guinea pig food. It is possible, therefore, that the preference shown for urine of guinea pigs fed GPF compared with urine of guinea pigs fed RF or synthetic food is the result of some aspect of previous experience with either guinea pig food or with urine from animals eating such food.

These data indicate that, in nature, male guinea pigs could discriminate among conspecific animals on the basis of previous dietary history. Although it is not surprising that dietary changes provide detectable differences in urinary chemical signals, the differential attractiveness of urine from animals on different diets shows that these differences have a communicative function. It is possible that information on the availability of a new food could be transmitted through urinary chemical signals. The importance of carefully controlling the diet in behavioural and biochemical studies of mammalian chemical communication is evident. Further, these data illustrate the complex nature of the information conveyed in guinea pig urine. In previous chemical studies, it has been found that separation of urine by a variety of procedures resulted in several fractions of widely different chemical character and molecular weight being responsible for its attractive nature of conspecific males⁹. It has been suggested² that a pattern of chemical substances may thus be involved in the urinary communication system of *Cavia* as well as in similar systems in other mammals. The data presented here further support these hypotheses.

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Differentiation induced by cyclic AMP in undifferentiated cells of early chick embryo *in vitro*

THE undifferentiated cells of the chick embryo in the post-nodal piece (PNP), obtained by a transverse cut at 0.5 mm posterior to Hensen's node of stage 4 blastoderm, remain incapable of differentiation when grown in a variety of media or by chorioallantoic grafting technique. When combined with the region anterior to the streak, including Hensen's node, however, the PNP would undergo normal differentiation similar to the embryonic axial structures^{1–3}, suggesting that information might have been transferred from one tissue to another. Later studies by Butros² and Niu and coworkers^{4,5} have shown that the PNP explants could be induced to develop into specific tissues, such as pulsating cardiac muscle tissues, when the explants were cultured in the presence of RNA isolated from the embryonic heart. The development was organ specific since RNA from kidney or thymus was incapable of inducing the heart formation⁵. To confirm this mode of differentiation in the PNP and to provide a meaningful biochemical interpretation to the phenomenon, we have begun studies examining the precise nature of the competent RNA and the sequence of events that leads to the formation of highly organised myofibrils. In a parallel study, we asked whether addition of exogenous cyclic AMP, which seems to be involved in control of morphological differentiation and biochemical changes in both normal and neoplastic cells (for review see ref. 6 and subsequent papers 7–15) would affect the development of the PNP in culture. Here we report that cyclic AMP, in the absence of exogenous RNA, can indeed produce specific morphological transformations, including the formation of heart-like pulsating tissues.

Fertile Leghorn chicken eggs from Spring Lake Farms, New Jersey, were incubated at 38 °C to obtain the definitive streak stage, stage 4 (ref. 16). The area opacua was trimmed off from the explanted blastoderms of the egg and the PNP was obtained by transecting the area pellucida at 0.6 mm behind Hensen's node to exclude totally the presumptive heart-forming region^{17,18}. The PNPs collected from 20–30 eggs per experiment, were pooled together, and then divided randomly in batches of 3 each for *in vitro* cultivation. When the explants were cultured in the absence of cyclic AMP (control) the growth pattern of PNP was similar to that described previously^{5,19}. The primitive streak disappeared after 24 h of incubation and patches of red blood cells appeared on the third day. No axial structures and twitching tissues were seen in any of the explants even after 10 d of incubation (Fig. 1a). The absence of twitching tissues in all the control explants (see Table 1) indicated

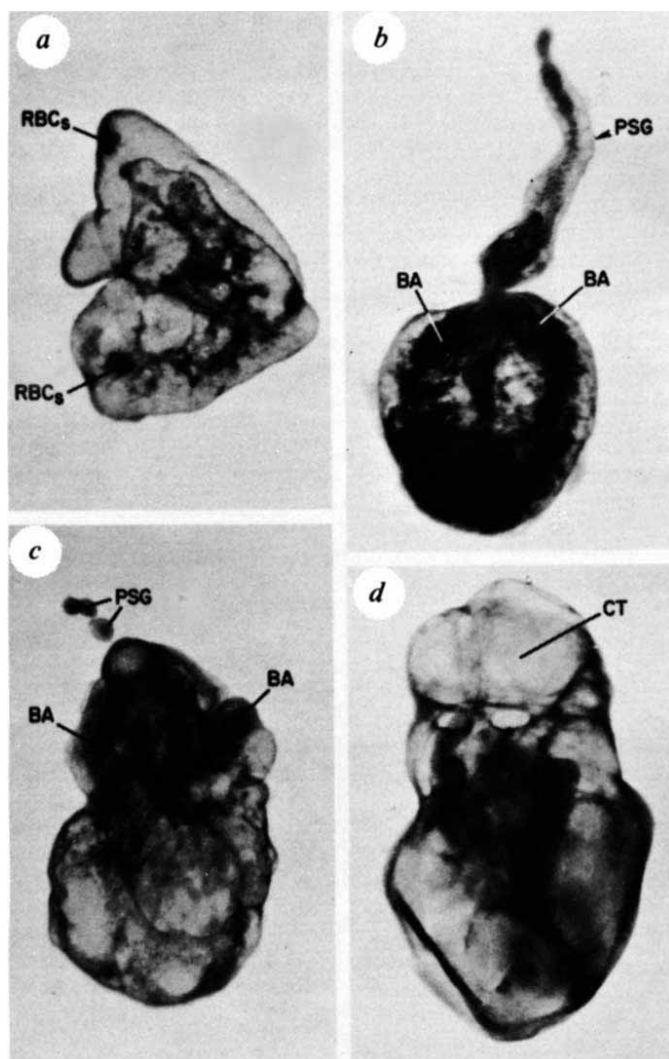


Fig. 1 Whole mounts of PNP cultured with or without cyclic AMP (0.5 mM). The cultivation was a modification of New's technique³¹ as described earlier³². Three PNPs were placed on the yolk-free vitelline membrane (VM) previously mounted around a glass ring, with the epiblast in contact with the VM. After removing excess PC (Pannett-Compton) solution³² the PNPs were carefully flattened on the VM. One millilitre of the nutritive medium (NM)¹⁹ was then pipetted in each dish containing 3 PNP. The medium contained 50 U ml⁻¹ of penicillin and 50 µg ml⁻¹ of streptomycin. The pH of the final solution after adding the supplements was 7.6. Cyclic AMP (0.5 mM) was added directly to the medium. Incubation was done at 38 °C and the PNPs were examined at regular intervals. All operations were carried out in sterile conditions. The PNPs were examined at regular intervals with a binocular microscope for changes in growth, appearance of the pulsating tissues and general morphology. The PNPs were photographed after staining with haematoxylin and eosin Y at times indicated. *a*, -cyclic AMP, 72 h incubation; *b*, + cyclic AMP 40 h incubation; *c*, + cyclic AMP, 72 h incubation; *d*, +cyclic AMP, 96 h incubation. RBCs, patches of red blood cells; PSG, primitive streak outgrowth; BA, beating area; CT, cardiac tube. Magnification ×30.

of the outgrowth projection, that is, the anterior end of the primitive streak, was similar for all the cyclic AMP-induced PNPs. Further incubation resulted initially in the appearance of infrequent twitchings near the basal portion of the outgrowth (Fig. 1*b*) which changed on day four, into rhythmic and spontaneous pulsations resembling that of cardiac tissues in normal intact embryos. This was accompanied by a progressive digression of the PSG, which was first transformed into a beaded string-like structure breaking off eventually from the rest of the explant (Fig. 1*c*). After 4 d of incubation cardiac tubular structures with rhythmic pulsations were visible (Fig. 1*d*). The steady rate of pulsations lasted 2–4 d without supplementing the medium. An important difference between the cyclic AMP-induced growth of the nodeless streak and that of normal intact embryo was the absence of normal regression of the primitive streak in the former.

An ultrastructural examination of the PNPs (Fig. 2) revealed that typical epithelial, mesenchymal and erythroblastic cells were found in explants of both control and experimental groups. Scantly-dispersed endoplasmic reticulum, vesicular nuclei and a few mitochondria appeared to be the characteristic features of the undifferentiated PNP. However, all randomly selected PNPs grown with effective concentrations of cyclic AMP (see below) contained, in addition to the normal components, highly differentiated myoblasts which seemed to be similar in ultrastructural complements to the normal chick embryonic myocardial cells. Clearly evident were the

that transection at 0.6 mm posterior to the node did exclude the entire presumptive heart-forming region from the PNP. In contrast, the addition of cyclic AMP (0.5 mM) caused striking changes in growth and development of the PNP. Not only did the primitive streak persist during the early stages, but it eventually grew to be equal in length to the whole explant, and in addition, a median structural outgrowth (PSG) projecting from the anterior end of the streak became visible during the first 24 h of incubation. The site

Table 1 Effect of cyclic AMP and analogues on differentiation in PNP

Additions	No. of PNP treated	No. of differentiated PNP*
Cyclic AMP (0.05 mM)	12	0 (0%)
Cyclic AMP (0.1 mM)	12	1 (8%)
Cyclic AMP (0.5 mM)	39†	29 (74%)
Theophylline (0.5 mM)	6	0 (0%)
Theophylline (1.0 mM)	6	0 (0%)
Theophylline (2.0 mM)	10	6 (60%)
Cyclic AMP (0.1 mM) + theophylline (0.5 mM)	6	4 (66%)
Dibutyryl cyclic AMP (0.05 mM)	3	0 (0%)
Dibutyryl cyclic AMP (0.1 mM)	3	0 (0%)
Dibutyryl cyclic AMP (0.5 mM)	9	2 (22%)
Dibutyryl cyclic GMP (0.5 mM)	6	0 (0%)
Adenosine (0.1 mM)	6	0 (0%)
Adenosine (0.2 mM)	6	5 (83%)
Adenosine (0.5 mM)	19†	15 (79%)
Adenosine (0.2 mM) + theophylline (1.0 mM)	6	0 (0%)
Adenosine (0.5 mM) + theophylline (1.0 mM)	6	4 (66%)
No additions (control)	84†	0 (0%)

Details of culture condition were described in the legend to Fig. 1.

*Differentiation was characterised by the appearance of spontaneous and rhythmic pulsations after 96 h incubation. In addition, characteristic myofibrillar structures were found in all randomly-selected pulsating explants when examined in the electron microscope. None of the explants of control series or those treated with non-effective agents contained myofibrils.

†The number of PNP explants represents a cumulative total of several experiments.

orderly arranged myofibrils with intercellular apical junctions or intercalated disks as described previously^{3,20}. These myofibrils were absent in the PNPs of control group and in those which did not differentiate into beating tissues. Measurements of the total protein content²¹ using three

PNP explants per assay, done in triplicate, revealed that each PNP of stage 4 blastoderm contained $9.0 \pm 1.0 \mu\text{g}$ protein, which increased to $21. \pm 0.7 \mu\text{g}$ after 4 d of incubation in the absence of cyclic AMP. However, the PNP which differentiated into beating tissues contained $30.6 \pm 1.3 \mu\text{g}$ per protein per explant. Thus, the formation of myofibrillar structures, the acquisition of spontaneous and rhythmic pulsations, the appearance of distinct morphological changes, such as the persistence of the primitive streak, and the increase in total protein content were all characteristic features of only those PNPs which received effective amounts of cyclic AMP and provided clear lines of evidence for a differentiative transition in the PNP.

Cyclic AMP, at 0.5 mM, was most effective, whereas concentrations below this level caused little or no differentiation (Table 1). Dibutyl cyclic AMP, on the other hand, when used at similar concentrations appeared to be a poor inducer. Theophylline, which acts specifically as an inhibitor of phosphodiesterase and thereby increases the intracellular level of cyclic AMP²², also induced differentiation similar in microscopical examination to that caused by cyclic AMP. Furthermore, a synergistic effect was clearly evident when non-effective concentrations of cyclic AMP (0.1 mM) and theophylline (0.5 mM) were added together to the culture medium. These results led us to speculate that changes in intracellular levels of cyclic AMP might be responsible for the specific morphogenetic transformations in the PNP.

Table 2 Endogenous levels of cyclic AMP in cultured PNP explants

Incubation Time (h)	Experiment	Cyclic AMP (Pmol μg^{-1} protein)			
		No additions	+cyclic AMP	+ADO	+THEO
0	1	0.019	0.023	0.020	0.026
	2	0.022	0.030	—	—
12	1	0.030	0.490	0.070	0.100
	2	—	0.460	0.066	0.088
48	1	0.058	0.730	0.040	—
	2	0.044	0.520	—	—
96	1	0.050	0.690	0.125	0.091

Endogenous levels of cyclic AMP were essentially determined according to Gilman³⁵ and Brown *et al.*³⁶ and as described in Amersham/Searle *Cyclic AMP Assay Kit*, TRK 432. Exogenous cyclic AMP, Ado (adenosine), or Theo (Theophylline), 0.5 mM each, were added directly to the culture medium (NM) and the PNPs were placed on top of the vitelline membrane (VM) as described in the legend to Fig. 1. At times indicated the explants were removed, washed 4 times with ice-cold Ringer's solution. Removal of extracellular cyclic AMP was indicated by no further decrease in cyclic AMP with additional washes. The PNPs were homogenised in 5% trichloroacetic acid. The precipitate recovered after centrifugation was used for protein determination²¹ and the supernatant was extracted thoroughly with ether and dried by lyophilisation. The dried material was taken up in Tris-EDTA buffer for cyclic AMP determination. Three PNPs were used per assay and the assays were done in duplicate for each experiment. The numbers above represent an average of six PNPs for each time point.

This assumption was tested by (1) a direct estimation of endogenous cyclic AMP levels and (2) by the exogenous addition of adenosine which is known to cause an elevation of cyclic AMP levels in a variety of tissues²³⁻²⁵, because of the direct activation of the enzyme adenylate cyclase by adenosine²⁶. Intracellular cyclic AMP level was estimated as described in the legend to Table 2. The explants after incubation with exogenous cyclic AMP were washed 4 times with ice-cold Ringer's solution. Removal of all extracellular cyclic AMP was indicated by no further decrease in cyclic AMP with additional washes. When cyclic AMP was added to the culture medium, there was a 25-fold increase in the intracellular cyclic AMP level after 12 h incubation (Table 2). This increase represented about 70% of the total increase observed at 48 h incubation, which was the saturation level of cyclic AMP uptake. The 12-h-old explants were almost one-third in size and in protein content compared with those

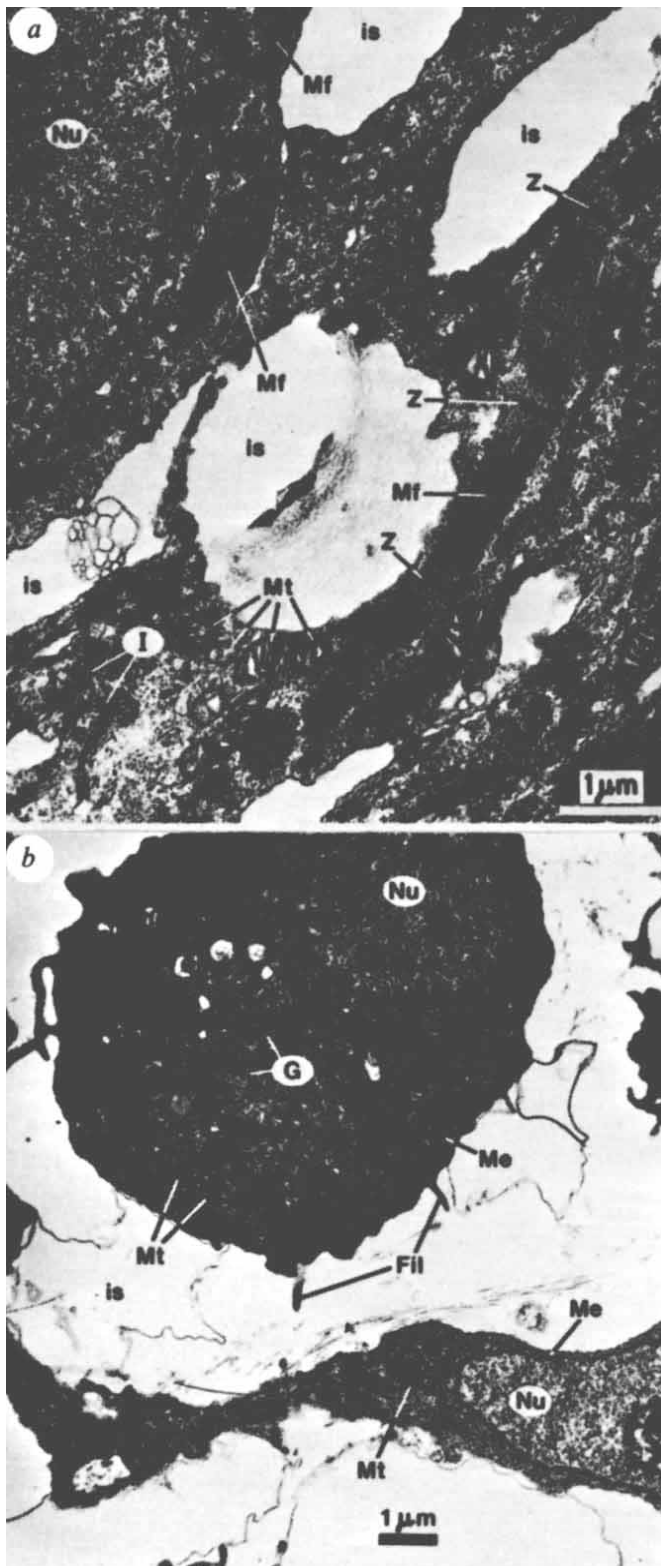


Fig. 2 Electron micrographs of cells of PNP after 96 h incubation with or without cyclic AMP. Electron microscopy was done in Dr M. Boublík's laboratory at the Roche Institute of Molecular Biology according to O'Brien *et al.*³⁴. *a*, +cyclic AMP (0.5 mM); *b*, —cyclic AMP; Nu, nucleus; Mf, myofibrils; Z, Z-bands; is, intercellular space; I, intercalated disk; Mt, mitochondria; G, Golgi body; Fi, filopodia; Me, mesenchyme.

cultured for 96 h. It would seem therefore that the uptake of cyclic AMP occurred predominantly during the early stages of incubation and was not directly related to the growth and increase in size of the explants.

Addition of theophylline and adenosine to the culture medium also caused a significant increase in the intracellular cyclic AMP levels (Table 2). The increase was, however, substantially lower than that observed with exogenous cyclic AMP, but was apparently sufficient to cause the differentiative transition in the PNP (see Table 1). A specific feature of the adenosine effect is the fact that it can be abolished by theophylline (methylxanthine), which acts as a competitive inhibitor of adenosine^{24,26,27}. As shown in Table 1, adenosine, but not guanosine, could mimic the effect of cyclic AMP. The concentration of adenosine required for an optimum effect was lower than that required for cyclic AMP. This might be due to a differential uptake of these compounds by the PNP in the culture conditions used. The adenosine-stimulated differentiation was blocked effectively by theophylline. The inhibition caused by theophylline was diminished when adenosine concentration was raised from 0.2 to 0.5 mM, indicating the competitive nature of these drugs. These effects of theophylline and adenosine on PNP were strikingly similar with those observed on cyclic AMP system in other tissues. Thus, the results taken together strongly suggested that the observed changes in the PNP growth and development were caused by modulations in intracellular levels of cyclic AMP.

The multiplicity of the effects caused by cyclic AMP was reflected by the formation of highly organised myofibrillar structures, acquisition of spontaneous pulsations and by the appearance of specific morphological changes described above. A recent report²⁸ has confirmed these findings and has shown that in addition to heart-like tissues, neural tissues, notochord and nephric tubules were also noted. The concentrations of cyclic nucleotides required, however, were 6–30-fold higher than those used in our studies. Reporter and Rosenquist²⁹ have previously reported that cyclic AMP levels in the precardiac mesodermal area in head-process stage of the chick embryo was about 20 times higher than in kidney-forming, liver mesoderm, and in neuro-epithelial ectoderm regions. The authors suggested that the regional differences in cyclic AMP concentrations may indicate the possible involvement of cyclic AMP, a presumed morphogen, in early chick embryonic development. We believe that there is a relationship between the state of development of the cultured PNP explants and the changes in cyclic AMP levels together with the cyclic AMP-associated properties of cells, such as the changes in membrane permeability assembly of microtubules, and so on. But the relationship between the exogenous RNA-induced mode of differentiation^{2,4,5,30} and that caused by cyclic AMP is yet to be ascertained.

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Postnatal development of the synaptic organisation of the lateral geniculate nucleus in the kitten with unilateral eyelid closure

CLOSURE of the eyelids over one eye during a well defined period of susceptibility in kittens has been shown to result in severe structural changes of the nerve cells in the lateral geniculate nucleus^{1,2}. Neurons in those parts of the lateral

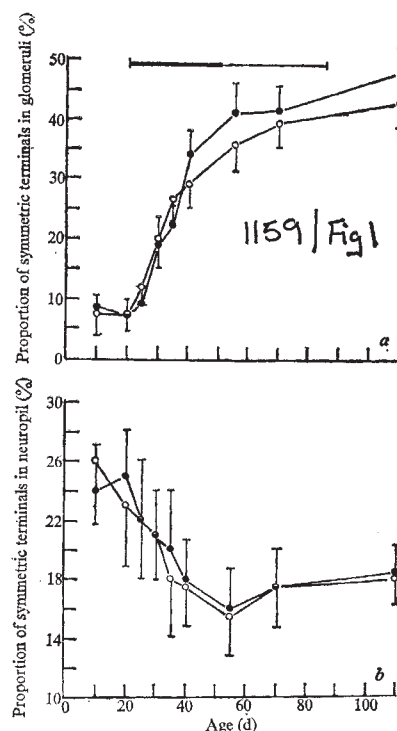


Fig. 1 The change in proportion of the symmetric synapses with age in the glomeruli (a) and the extralaminar neuropil (b) of the binocular parts of lamina A and A1 of the lateral geniculate nucleus in the normal kitten. The phase of rapid change occurs during the period of maximum susceptibility. Vertical bars indicate standard error; ●, lamina A, ○, lamina A1; thick part of the horizontal bar indicates period of maximum susceptibility and thin part the period of declining sensitivity.

geniculate nucleus which are related to the binocular visual field and which receive fibres from the closed eye are smaller than those in the laminae innervated from the open eye. The fact that cellular changes do not occur in the part of the nucleus related to the monocular visual field has led to the hypothesis that there is a process of competition or an imbalance between the neurones in the deprived and stimulated portions of the binocular parts of the laminae, whereas such competition is not present in the monocular segment^{2,3}. We have examined the lateral geniculate nucleus with the electron microscope at different intervals extending through the period of susceptibility after monocular eyelid closure and in a parallel series of normally reared kittens.

In nine kittens the eyelids over one eye were sutured together at the time of eye opening at about 10 d of age, and these animals were allowed to survive for varying periods, the longest survival period being 100 d. These animals were perfused at 24 °C with a mixture of 4% paraformaldehyde and 1% glutaraldehyde after a brief washout with a balanced salt solution. The lateral geniculate nucleus of both sides was removed from the thalamus, cut coronally into three blocks and processed for electron microscopy. After examination of 1- μ m thick sections stained with toluidine blue⁴, ultrathin sections were cut including the optic radiation and adjoining parts of laminae A and A1 and from the monocular segment of lamina A. In all animals the sections were taken from the middle third of the anteroposterior extent of the nucleus, from about the middle of the mediolateral extent of laminae A and A1 and from the monocular segment² in the lateral part of lamina A.

A study of the monocularly deprived lateral geniculate nucleus with the electron microscope showed that the de-

prived laminae underwent little qualitative change compared with the undeprived laminae, and as the differences in cell sizes are not so apparent in ultra-thin sections it was often not possible to determine which were the deprived and undeprived laminae. There was no change in the appearance of the cell somata and the constituent organelles, apart from a slight diminution in the amount of the granular endoplasmic reticulum, in the dendritic profiles, axons, axon terminals and glia; in particular the large retinal terminals showed no abnormalities either in their electron density or synaptic vesicle content. Thus, there is no evidence for degeneration either of the axons from the deprived retina, or of the constituent neurones of the nucleus, nor was there a glial reaction. In view of this unexpected absence of qualitative changes a quantitative study was made, and because of the evidence suggesting that the cells of the undeprived laminae in such experimental animals may have undergone some hypertrophy^{3,5,6}, it was considered necessary to compare the quantitative data in this series with corresponding data from the laminae in normal kittens of the same ages. The brains of nine normal kittens were therefore perfused in the same manner and ultra-thin sections for electron microscopy taken from corresponding parts of the lateral geniculate nucleus.

Within the glomeruli the numbers and proportions of the asymmetric and symmetric synapses were counted, and their postsynaptic profiles were recorded at each age. No attempt was made to differentiate between the symmetric contacts formed by axon terminals and presynaptic dendrites. About 25 glomeruli were sampled randomly from the binocular parts of each of the laminae of the lateral geniculate nucleus of both sides in the experimental brains, and of one side of the normal brains, and similar data from the monocular segments of lamina A were obtained. Between

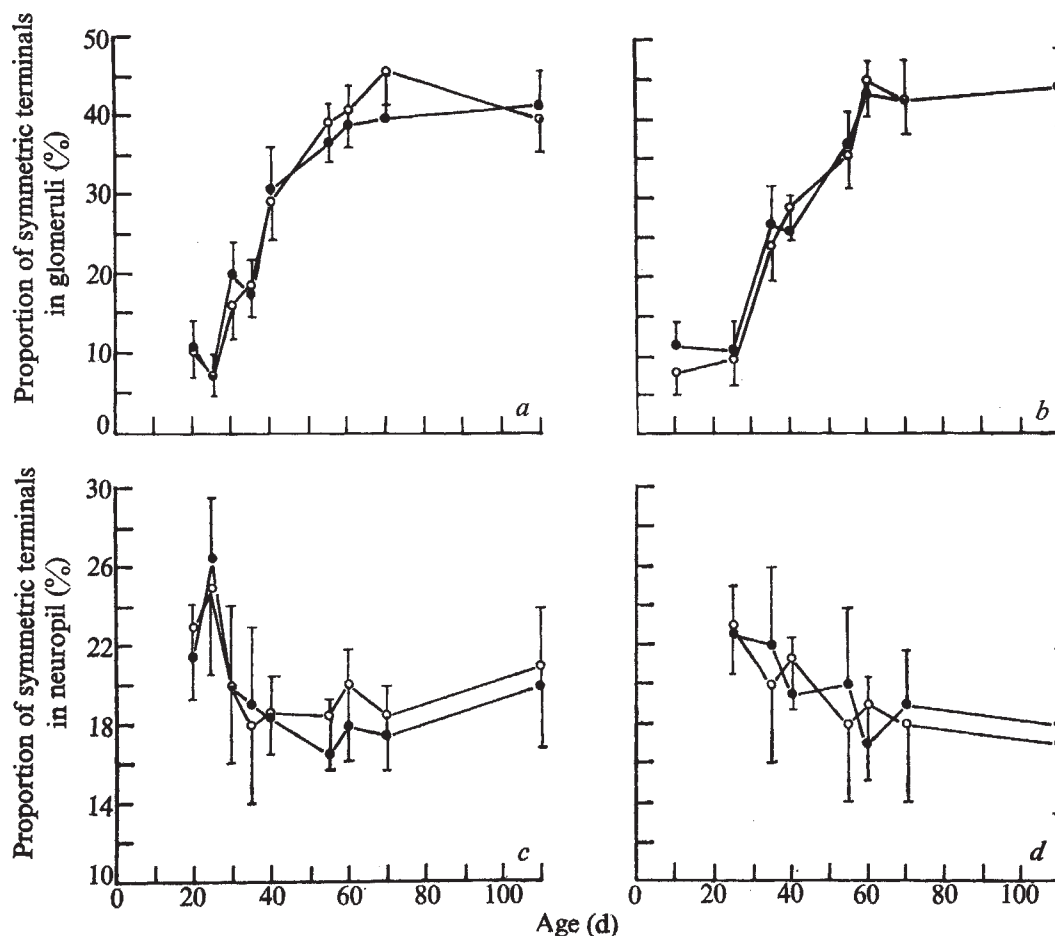


Fig. 2 The change in proportion of symmetric synapses with age in the glomeruli (a and b) and in the extraglomerular neuropil (c and d) of the binocular segments of laminae A and A1 of the ipsilateral (a and c) and contralateral (b and d) lateral geniculate nucleus after monocular eyelid closure. On the ipsilateral side lamina A1 has been deprived, and on the contralateral side lamina A. ●, lamina A; ○, lamina A1.

100 and 150 synapses were classified into these categories in each lamina, the total sample amounting to approximately 10,000 synapses. These counts were made from micrographs at a magnification of 16,000. The proportions of these two types of synapses were also estimated in the extraglomerular neuropil, approximately the same number being sampled as in the glomeruli; they were classified at the time of study on the microscope.

Within the glomeruli of the normal kittens there is a phase of rapid increase in the proportion of the symmetric synapses from less than 10% at 20 d to between 35 and 40% at 55 d, with relatively little further change up to 110 d (Fig. 1a). In contrast, in the extraglomerular neuropil, there is a steep fall in the proportion of symmetric synapses from 25 to 15% between 20 and 55 d (Fig. 1b). These figures confirm the qualitative observations that between 20 and 55 d there is a differentiation of the profiles within glomeruli, especially an increase in the number of vesicle-filled presynaptic dendrites making symmetrical synapses; during this period the glomeruli also became more clearly segregated by their glial envelopes. The time course and degree of maturation finally attained is similar in both main laminae. The comparable data from the glomeruli and the neuropil from the experimental brains show no significant (two-tailed *t* test, $P > 0.05$) differences in the rate of development between the laminae in the normal and experimental brains and between the deprived and undeprived laminae on the two sides of the same experimental brains (Fig. 2). The

in distinguishing between relay and presynaptic dendrites at earlier ages when the presynaptic dendrite is not so clearly differentiated. Similar comparisons cannot be made about the profiles postsynaptic to symmetric synapses because the number of presynaptic dendrites making synapses at younger ages is very small, and they are less definitely identifiable, but at 110 d the majority of the symmetric synapses, like the asymmetric synapses, are on the relay cell dendrites. No statistically significant differences ($P > 0.05$) were found in the postsynaptic profiles between the monocular and binocular segments, nor between the laminae of normal brains and the undeprived and deprived laminae of the brains of the experimental animals of the same ages.

The numerical data used for the estimates of the proportions of the types of synapses and of the postsynaptic profiles have also been used to provide estimates of the mean numbers of synapses per glomerulus at different ages. These data confirm the qualitative impression that the number of symmetric synapses in a glomerulus increases with age (for example the mean number at 20 d is 0.33 (s.e. 0.47), whereas at 110 d it is 2.76 (s.e. 1.76) in lamina A; $P < 0.001$). The mean number of retinal synapses does not show a significant change with age, so the explanation for the change in the proportions of the synapses that has already been noted is due mainly, and perhaps solely, to an increase in the number of symmetric synapses. The brains of the experimental series showed similar changes with age and significant differences ($P > 0.05$) were not found between them and those of the normal brains.

These electron microscopic observations show that there is a marked degree of development of the synaptic organisation occurring during the period of susceptibility to effects of eyelid closure, but that there are no appreciable differences to be found between the nucleus in a normal brain and in those animals subjected to monocular eyelid closure. Thus, if the shrinkage of the neurones in the lateral geniculate nucleus seen with the light microscope is associated with alterations in the retinal terminals or in the synaptic organisation, it must be at a level more subtle than that studied here. The cell shrinkage may be related to features of the intrinsic organisation, such as reciprocal and serial synapses, or to changes in the number of synaptic vesicles within terminals. It may, on the other hand, not be related to change in the lateral geniculate nucleus, but at the site of termination of the geniculocortical fibres in the visual cortex^{7,8}. The findings in the series of normal brains of a marked change in the proportions of synapses within glomeruli and the extraglomerular neuropil between the ages of 20 and 55 d is further evidence that the nucleus is undergoing a phase of rapid morphological development during the period of susceptibility to monocular deprivation. It has already been shown that during this period there is a rapid growth in the size of cell bodies within the nucleus⁹, in the synaptic density¹⁰, and in the degree of myelination of the incoming optic tract fibres¹¹ (Fig. 3). It is of interest that within the glomeruli the change in proportion is due to an increase in the number of symmetric terminals, which, on present evidence, arise from interneurons^{12,13}, whereas in the neuropil the asymmetric synapses increase relatively more and these are probably predominantly the terminals of cortico-geniculate axons and collaterals of geniculocortical relay cells^{14,15}. A change, however in the proportion of intrinsic axons after monocular and binocular eyelid suture has been found in another major site of termination of retinal fibres, the superior colliculus in the rat, where the final phase of growth of intrinsic axons fails to occur after eyelid closure¹⁶. This presents a paradox that within the superior colliculus unilateral eyelid closure has an effect on the attainment of the mature synaptic organisation, although no appreciable shrinkage of cells has been found, yet in the lateral geniculate nucleus, where there is a marked shrinkage of the neurones, there does not seem

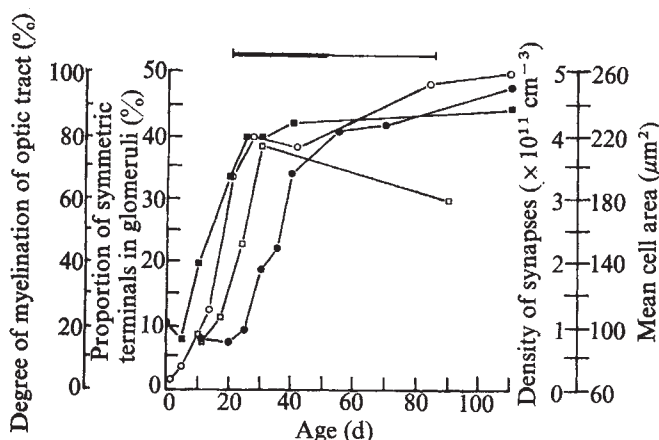


Fig. 3 A comparison of the time course of the change in proportion of symmetric synapses (●) with the other morphological changes which occur in lamina A of the lateral geniculate nucleus and optic tract during postnatal development in the kitten. Cell size (□) from Garey *et al.*⁹, synaptic density (■) from Cragg¹⁰ and myelination of optic tract fibres (○) from Moore *et al.*¹¹. Horizontal bar indicates period of susceptibility.

proportions of the two types of synapses within the glomeruli and the neuropil of the monocular segment follow the same course of development as the binocular segments and there are probably no significant differences ($P > 0.05$) in the deprived and normal monocular segments.

An analysis has been made of the types of profiles postsynaptic to the asymmetric and symmetric synapses respectively within the glomeruli. At younger ages there are more relay cell dendrites receiving asymmetric synapses than presynaptic dendrites (for example, at 20 d 90% of the profiles postsynaptic to the asymmetric retinal boutons are relay cell dendrites), but in progressively older animals there is an increasing number of presynaptic dendrites receiving such synapses (at 70 d about 70% of the postsynaptic profiles are relay cell dendrites). This difference, however, may be more apparent than real because of the greater difficulty

to be any accompanying change in the synaptic organisation.

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Partial replacement of serum by selenite, transferrin, albumin and lecithin in haemopoietic cell cultures

CULTURE media for mammalian cells usually require supplementation with serum to supply as yet undefined needs. Because the requirements are likely to be multiple, it is difficult to distinguish biologically between the effects of nonspecific 'nutritional' factors in serum and those of specific regulatory factors. Partial replacement of serum in these systems by chemically defined substances supplying the nonspecific needs would therefore represent a significant advance towards the definition of such specific factors. We have examined the role of several serum components in cultures of freshly explanted haemopoietic cells with this goal in mind.

Red-cell precursors in freshly explanted mammalian bone marrow will proliferate to form colonies if the medium contains serum and the glycoprotein hormone erythropoietin^{1,2}. Similarly, colony formation by granulocyte/macrophage precursors is dependent on serum as well as a specific glycoprotein colony-stimulating factor³. We decreased the concentration of serum in these cultures until growth was limited. The serum concentration was made the only variable by maintaining erythropoietin or colony-stimulating factor at high and non-limiting levels. A mixture of a large number of known serum constituents was added to the cultures and restored growth. Elimination of the components one by one then established which were active and which were not. Four components—sodium selenite, transferrin, bovine serum albumin (BSA) and lecithin—accounted for all the activity of the mixture. We demonstrate here that in combination these substances replace most of the serum required for granulocyte/macrophage and erythroid colony growth and also facilitate some granulocyte/macrophage proliferation without added serum.

Bone marrow cells from the femurs of BDF₁ mice were plated in modified Dulbecco's medium (legend Fig. 1) made viscous with methyl cellulose². Erythroid colonies of eight or more cells were identified² after 2 d of incubation, and granulocyte/macrophage colonies of more than 100 cells were scored after 8 d.

In these conditions, maximum numbers of erythroid colonies required the addition of 30% foetal calf serum. When the serum concentration was decreased to 1%, no colonies formed (Table 1). At this serum concentration, selenite, BSA and transferrin added individually had little

effect, while in combination they stimulated the formation of large numbers of colonies. Addition of lecithin to this combination caused no further enhancement. Omission of either selenite, BSA or transferrin from the mixture resulted in a significant decrease in colony growth, while omission of serum almost completely abolished growth.

Maximum numbers of granulocyte/macrophage colonies ordinarily required the addition of 10-15% serum. When

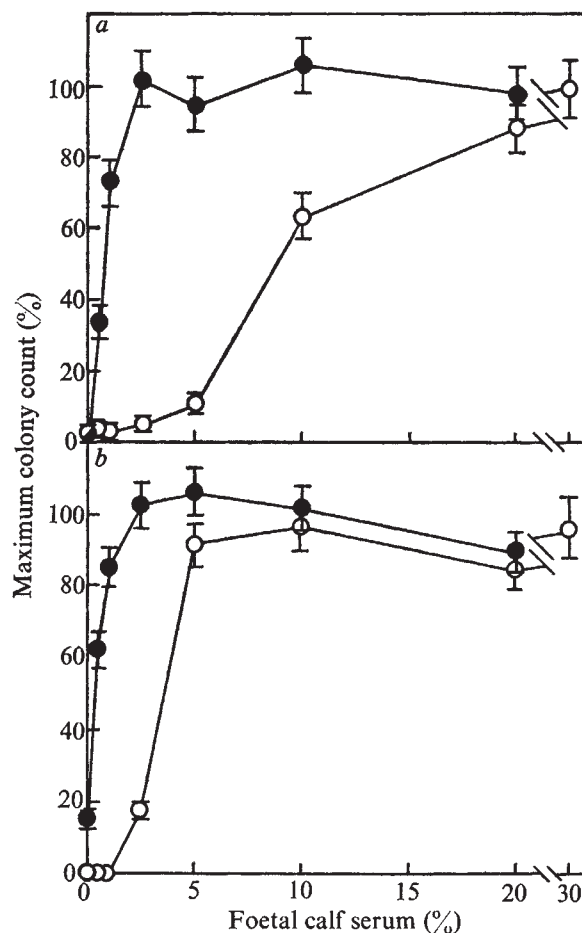


Fig. 1 Dependence of colony formation on foetal calf serum concentration. *a*, Erythroid colonies, percentage of counts at 30% serum (240 per 10⁵ cells), 0.5 erythropoietin units per ml. ○, Nothing added; ●, supplemented with 0.75% BSA, 3.4 × 10⁻⁸ M transferrin + 1.6 × 10⁻⁶ M FeCl₃, and 10⁻⁷ M Na₂SeO₃. *b*, Granulocyte/macrophage colonies, percentage of counts at 30% serum (126 per 10⁵ cells), plateau amounts of colony stimulating factor. ○, Nothing added; ●, supplemented with BSA, transferrin/FeCl₃, Na₂SeO₃ and 3 × 10⁻⁵ M egg lecithin. Bars indicate standard errors. Cells were cultured at 10⁵ per ml in Dulbecco's modified Eagle's MEM (Gibco H21) containing 0.8% methyl cellulose and supplemented with 10⁻⁴ M α-thioglycerol plus the following (μg ml⁻¹): L-alanine (25), L-asparagine.H₂O (50), L-aspartic acid (30), L-cysteine (70), L-glutamic acid (75), L-proline (40), Na pyruvate (110), vitamin B₁₂ (0.025) and biotin (0.03). Human urinary erythropoietin was adsorbed on to benzoic acid and further purified on DEAE cellulose⁴ and Sephadex G-100 (ref. 2). Colony-stimulating factor from serum-frec mouse kidney cell conditioned medium⁵ was partially purified by two passages over Sephadex G-150. BSA (Behringwerke, electrophoretically pure) was deionised over a mixed bed ion-exchange resin⁶, charcoal (Norit A) extracted⁷ (pH 3, 55 °C, 30 min) and chromatographed on an Aca 34 gel column to isolate the monomeric form. Human transferrin (Behringwerke) was further purified on Aca 34 and prepared as a 125 × stock (38 mg lyophilised transferrin + 48 μg FeCl₃.6H₂O per ml) in Dulbecco's medium. Na₂SeO₃ (Merck) was prepared as a 125 × stock solution (2.2 μg ml⁻¹) in Dulbecco's medium containing 5% BSA. Egg lecithin (BDH, > 95%) was dissolved in propylene glycol (25 mg ml⁻¹) and diluted 10-fold to a 125 × stock solution in Dulbecco's medium containing 5% BSA. Plate concentrations of propylene glycol (0.08%) were not inhibitory. Foetal calf serum was used without treatment.

Table 1 Effect of BSA, transferrin (Tf) and selenite (Se) on erythroid colony counts

Addition	Colonies per 10 ⁵ nucleated cells
30% Foetal calf serum (FCS)	353 ± 13*
1% FCS	0
1% FCS, Se	6 ± 3
1% FCS, BSA	13 ± 5
1% FCS, Tf	4 ± 2
1% FCS, Se, BSA, Tf	232 ± 9
—, Se, BSA, Tf	7 ± 3
1% FCS, —, BSA, Tf	117 ± 6
1% FCS, Se, —, Tf	42 ± 7
1% FCS, Se, BSA, —	67 ± 10

Concentrations of BSA, Tf and Se were as in Fig 1.

*Standard error.

serum was eliminated, no colonies formed (Table 2). Selenite, BSA, transferrin or lecithin had no effect when added individually to the serum-free medium. When added in combination, however, they stimulated growth of significant numbers of colonies. Although the colonies obtained in these conditions were generally smaller than those obtained with 15% serum, many contained at least 1,000 cells and were visible to the unaided eye. Omission of either BSA or transferrin from the mixture eliminated colony formation entirely and colony numbers were also reproducibly lower in the absence of selenite or lecithin.

The serum-sparing effect of these substances is demonstrated in Fig. 1. The addition of selenite, BSA and transferrin reduced the serum required for maximum numbers of erythroid colonies from 30 to 2.5%. With the further addition of lecithin, the serum requirement for maximum granulocyte/macrophage colony growth was reduced from 10 to 2.5%. Again it was interesting that whereas erythroid colony formation was dependent on serum even in the presence of these substances, some granulocyte/macrophage colony formation was possible in the absence of serum.

Table 2 Effect of BSA, Tf, Se and egg lecithin (lec) on granulocyte-macrophage colony counts

Addition	Colonies per 10 ⁵ nucleated cells
15% FCS,	146 ± 9*
None	0
Se	0
BSA	0
Tf	0
Lec	0
Se, BSA, Tf, lec	57 ± 4
—, BSA, Tf, lec	46 ± 7
Se, —, Tf, lec	0
Se, BSA, —, lec	0
Se, BSA, Tf, —	24 ± 3

BSA was used in a concentration of 1.2%.

Concentrations of Tf and Se were as for Fig 1.

*Standard error.

It is possible that the activities demonstrated for the proteins BSA and transferrin were attributable to trace impurities. Efforts were made to diminish this possibility by further purification of supplied materials (legend Fig. 1). Transferrin activity was not replaced by the equivalent amount of Fe³⁺ alone, nor was its activity reduced after gel permeation chromatography. Acrylamide slab gel electrophoresis of purified BSA and transferrin in denaturing conditions (0.1% SDS, 0.1% β-mercaptoethanol, 2 μg of protein per slot) revealed only a single Coomassie brilliant blue-stainable band in both instances.

We anticipate that the active substances reported here will prove to have similar nonspecific, serum-replacing activity in cultures of various other cells and tissues. Selenium, present in serum at a concentration of

1.4 × 10⁻⁷ M (ref. 9), has long been recognised as an essential trace element in whole animal studies¹⁰, and McKeehan *et al.* demonstrated it to be a requirement for growth of human fibroblasts in culture¹¹. The selenium-containing enzyme glutathione peroxidase is known to be present in leukocytes¹² as well as erythrocytes¹³. The requirement for transferrin by maturing red cells is not surprising, since this iron-transporting protein, present in serum at 4 × 10⁻⁵ M (ref. 14), is known to be the major source of iron for these cells. It was unexpected, however, that transferrin should also prove necessary for growth of granulocyte/macrophage precursors. Taken together with the observations that transferrin has a role in the proliferative response of human lymphocytes to PHA¹⁵, and in the growth of fibroblasts and pituitary cells in culture¹⁶, the finding suggests a much wider requirement for this protein than has been recognised. Among numerous conceivable functions of albumin, we consider the likeliest to be that of a buffer for components of the medium present in inhibitory amounts. This suggestion predicts that the albumin requirement would diminish if the numerous defined components of the culture medium were adjusted to optimum levels.

The straightforward interpretation of our observations is that a major role of serum in culture is to provide selenium, transferrin, albumin and lipids to the medium. Our results do not, however, exclude the possibility that these entities simply substitute for other unrelated substances provided by serum. Several agents that were expected to show activity did not enhance colony formation at low serum concentrations, provided that selenite, transferrin, albumin and lecithin were present and that erythropoietin and colony-stimulating factor were maintained at non-limiting levels. Among those tested were dexamethasone (2.5 × 10⁻¹⁰ M), insulin (2.5 × 10⁻⁸ M), testosterone (10⁻⁹ M), etiocholanolone and fluoxymesterone (3 × 10⁻⁷ M), prostaglandins E₁, E₂ and F_{2α} (5 × 10⁻⁹ M), ZnCl₂ (10⁻⁷ M), and cholesterol (3 × 10⁻⁷ M).

Our observations have important technical implications. First, inclusion in culture medium of the active substances reported here greatly reduces the quantities of serum required, and in our experience has transformed several serum lots from inadequate to fully active. More important, we expect their routine inclusion in medium to simplify the search for more specific biologically active substances in conditioned media and serum by reducing the number of variables in the system.

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Cell contact induces an increase in pinocytotic rate in cultured epithelial cells

ENDOCYTOSIS is the process by which cells internalise part of the plasma membrane in association with extracellular components: they engulf large particles by phagocytosis and internalise components in the fluid phase by pinocytosis. Discrimination of phagocytosis from pinocytosis has been based on the size of the particle and the amount of energy required to internalise it, phagocytosis being inhibited more readily by metabolic poisons than pinocytosis¹⁻³. This is consistent with the view that more energy is required to internalise a large surface area than is needed for a small surface area. Vasiliev *et al.*⁴ described "contact inhibition of phagocytosis": when cultured epithelial cells, but not fibroblasts, became confluent, the rate of phagocytosis decreased considerably. I report here that confluent cultured epithelial cells are not inhibited in their ability to undergo pinocytosis, but exhibit a greater rate of pinocytosis than do non-confluent cells. This difference is not due to diffusible factors, but is the result of cell contact.

Permanent lines of cultured epithelial cells of human (HeLa) or monkey origin (Mk₂) were grown as monolayers on plastic tissue culture dishes and maintained in Eagle's MEM containing 10% calf serum, penicillin (5 U ml⁻¹) and streptomycin (2 U ml⁻¹). Cells were passaged routinely every 4-5 d. Pinocytosis was measured using horse-radish peroxidase (HP) according to the method of Steinman *et al.*^{5,6}, or ¹²⁵I-albumin by Rhyser's procedure⁷. HeLa cells took up HP linearly with respect to both concentration (not shown) and time (Fig. 1). The level of endogenous peroxidase activity in HeLa cells was essentially unmeasurable (<0.1 ng enzyme per mg cell protein), and washing removed all non-internalised enzyme. These results support the conclusions of Steinman *et al.*^{5,6} on the value of HP as a pinocytotic marker. I also observed that when the uptake of enzyme was calculated on either a per protein (Fig. 1a) or a per cell basis (not shown), cultures of HeLa cells plated at higher densities exhibited greater rates of uptake than those of lower densities. In spite of slight differences in the

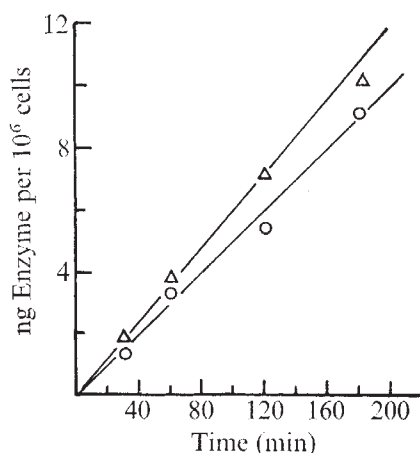


Fig. 1 Cultures of different numbers of HeLa cells were seeded 48 h before the experiment. Cells were then incubated in complete medium containing HP (1 mg ml⁻¹) for the times specified. After incubation the monolayers were washed eight times with cold phosphate-buffered saline (PBS), incubated in PBS at 4 °C for 20 min and washed four more times. The monolayers were then solubilised with 0.1% Triton X-100 and HP was assayed as before⁵. Duplicate cultures were trypsinised and cells were counted. Protein concentrations were determined by the procedure of Lowry *et al.*¹⁰. Each point is the average of triplicate samples. The standard error of the mean is 5-8% for both cell number and enzyme assay. Δ , 1.25×10^6 cells per plate; $\circ$, 0.75×10^6 cells per plate.

absolute amount of HP taken up in different experiments (10-20%), there was a consistent difference in the rate of uptake between cultures seeded at different densities. This is demonstrated more strongly in Fig. 2, in which a wider range of cell densities was used. Similar differences in the rate of uptake were observed whether marker was presented to cells in fresh medium or medium conditioned by exposure to confluent cells for 3 d.

Mk₂ cells are derived from a pool of normal rhesus monkey kidneys, and unlike HeLa cells, they have several characteristics typical of normal cells, such as density-dependent inhibition of growth and macromolecular synthesis, and an inability to grow in either suspension or soft agar⁸. I also observed that as Mk₂ cultures became more dense, the rate of pinocytosis increased, and stabilised only as they reached their saturation density (data not shown). Similar results were obtained if pinocytosis was assessed on a per cell instead of a per protein basis. Thus, a density-dependent increase in the rate of pinocytosis is not unique to HeLa cells.

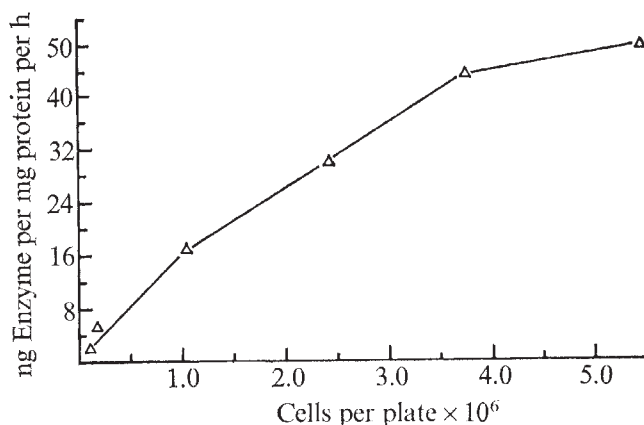


Fig. 2 Uptake of HP by different densities of HeLa cells. Different numbers of HeLa cells were seeded on plates 72 h before the experiment. Monolayers were incubated in medium containing HP (1 mg ml⁻¹) for 3 h and then processed as described for Fig. 1. Duplicate samples were taken and cells were counted.

The difference in rate of uptake was independent of HP. When iodinated albumin was used as a pinocytotic marker, cultures of HeLa or Mk₂ cells at a higher density exhibited a greater rate of uptake than those at a lower density.

To establish that the difference in accumulation of pinocytotic markers was the result of a difference in uptake, I measured the rate of intracellular inactivation of HP. Cultures of HeLa cells plated at different densities and exhibiting a twofold difference in uptake inactivated the enzyme at the same rate (Fig. 3). In both cases, the enzyme was inactivated with single exponential kinetics (correlation coefficient >95%), yielding a half life of 6.5 h. HeLa cells plated at different densities degrade ¹²⁵I-albumin at similar rates, yielding a half life of 16.4 h. Thus, differences in the rate of accumulation reflect differences in the rate of uptake rather than degradation.

The progressive increase in the rate of pinocytosis as cell density increased (Fig. 2) was independent of cell number but not cell density as the following experiment showed. Using small glass cylinders of different diameter, I plated a constant number of cells per dish. This procedure yields different cell densities, thus modifying the degree of cell-cell contact. I found that an increase in cell density gave rise to an increase in pinocytosis regardless of whether the pinocytotic marker was presented to cells in fresh medium or medium conditioned by exposure to confluent monolayers. Thus, an increase in the degree of cell contact induced an increase in the rate of pinocytosis.

As the difference between rates of uptake by cells plated at different densities was the same in fresh and conditioned

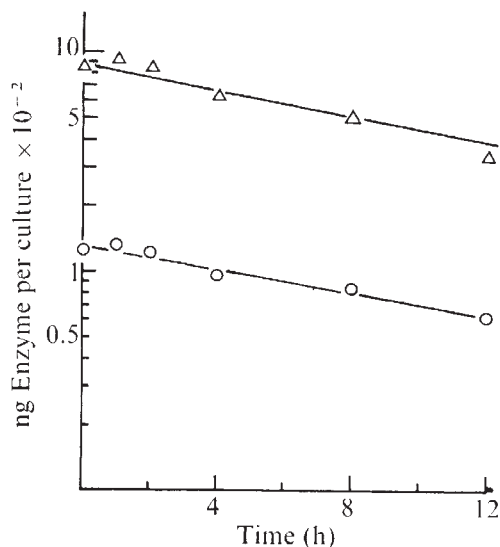


Fig. 3 Rate of degradation of internalised HP. Monolayers of cells were incubated with HP (1 mg ml^{-1}) for 20 h and then washed six times with cold MEM and twice with warm MEM + 10% calf serum. At the times specified, monolayers were collected and enzyme was assayed as described for Fig. 1. The symbols represent experimental data, the line is a computer-determined best fit to the data. At the end of the experiment the cultures had densities of $1.41 (\Delta)$ and $3.81 \times 10^6 (\circ)$ cells per plate.

medium, I suggest that diffusable factors are not responsible for the difference in the rate of pinocytosis. I found that if cellular levels of cyclic AMP were increased by addition of dibutyryl cyclic AMP (1 mM) or caffeine (10 mM), the rate of pinocytosis in sparse cultures was not affected.

The change in rate of pinocytosis in HeLa and Mk_2 cells occurs before any overt change in growth rate. For example, Mk_2 cells divide at a constant rate with a generation time of 42 h until a density of 5.5×10^5 cells per cm^2 is reached⁸. HeLa cells divide at a constant rate until a cell density of 1.2×10^6 cells per cm^2 is reached, after which the rate of division decreases by 80–90% (my unpublished results). But before any change in growth rate, the rate of pinocytosis may change by a factor of four to six. This suggests that changes in the rate of pinocytosis are independent of inhibition of cell division.

The cell types used in this study were of epithelial origin. It has been demonstrated, however, that L cells⁶ and sarcoma 180 cells (H.J.P. Rhyser, personal communication) of fibroblast origin exhibit a density-induced increase in pinocytosis. Apart from the Mk_2 cell all these cells are derived from established tumours. Although Mk_2 has several characteristics typical of normal cells, it has been maintained in culture for 16 yr; perhaps the density-dependent increase in pinocytosis results from adaptation to tissue culture. I am investigating whether primary cultures of epithelial cells exhibit a density-dependent increase in pinocytosis.

Using either polystyrene beads ($1.10 \mu\text{m}$ in diameter) or Oil red O-albumin emulsions, I have not been able to quantify the rate of phagocytosis in either non-confluent or confluent monolayers incubated for up to 4 h. Thus I cannot comment directly on "contact inhibition of phagocytosis". It may be that the difference in phagocytosis is related to a change in the adhesive characteristics of the membranes of confluent cells, as suggested previously^{4,9}. Whether or not "contact inhibition of phagocytosis" can be confirmed, my results show that it is not due to the inability of the cell to internalise plasma membrane. Contrary to expectation, the occurrence of cell-cell contact seems to enhance at least one membrane function.

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Presence of membrane particles in freeze-etched bovine olfactory cilia

WHEN an odorant molecule stimulates an olfactory receptor cell the electrical changes which are set up must result from some kind of interaction between the stimulant and the cell surface. Various theories of olfaction have favoured either lipids¹ or proteins², as the principal site of odorant reception. Experiments distinguishing unequivocally between these alternatives have yet to be performed³, but there is growing evidence that the receptor is proteinaceous^{4,5}.

Two other protein-containing receptors that have been extensively explored are rhodopsin⁶ and the acetylcholine receptors of skeletal muscle⁷ and electroplax origin⁸. All of these have been visualised in freeze-fractured preparations by electron microscopy as being related to particles 8–12 nm



Fig. 1 Freeze-etch replica of the surface of the bovine olfactory epithelium showing two receptor endings (re) and the apex of a supporting cell (sc). Note the broken basal portions of cilia at the surface of the sensory ending on the right, and two vertically cleaved cilia on the left ($\times 25,000$).



Fig. 2 Detail of Fig. 1 showing membrane features of two sensory cilia. In the cilium on the right, numerous membrane particles and part of a ciliary necklace (arrow) are visible on the exposed PF face. In contrast the EF face shown in the cilium to the left, bears few or no membrane particles ($\times 90,000$).

across, embedded in the cell membranes of the sensory surfaces. If the olfactory receptor sites are indeed of a similar nature, one also might expect to find membrane particles in the sensory cilia of olfactory receptor cells, but until now attempts to observe them have been unsuccessful⁹⁻¹¹. In this letter we report the discovery of high densities of membrane particles in the sensory cilia of bovine olfactory cells.

Heads of male calves (1 month old) were longitudinally bisected and olfactory tissue on the cribriform plate and ethmoturbinates fixed *in situ* with ice-cold Karnovsky's fixative¹² buffered at pH 7.0, for 1 h, then excised and placed in fresh fixative for 2 h. Tissue from the neighbouring ciliated non-sensory respiratory mucosa was also treated in the same way for control purposes. After washing in buffer, small pieces of tissue were soaked in 20% cacodylate-buffered glycerol (pH 7.0) containing 0.01% CaCl_2 for 24 h and were then fractured, etched and replicated in a Balzers GA-6 freeze etching device according to standard techniques¹³. Replicas were observed in an Hitachi 125E electron microscope at 125 kV. The nomenclature recently proposed by Branton *et al.*¹⁴ will be used in this description. In the figures, shadows appear white.

In addition to studies of freeze-etched material, pieces of tissue were also examined in sections by routine transmission and scanning electron microscopy for comparison with the freeze-etched material. In this way, the olfactory and non-olfactory tissue could be unequivocally distinguished in both types of preparation.

The general structure of the sensory tissue of the ox is similar to that of other mammals which have been studied with the electron microscope¹⁵. The surface of the olfactory epithelium is formed partly by the terminal knobs of sensory dendrites bearing an average of 17 cilia which possess short proximal portions averaging $1.7 \mu\text{m}$ long by $0.2 \mu\text{m}$ wide, tapering to long trailing distal regions $0.08 \mu\text{m}$ in diameter and at least $30 \mu\text{m}$ long; these distal portions are expanded

occasionally to form vesicles averaging $0.6 \mu\text{m}$ across. The supporting cells surround and separate the receptor cells, and bear at their apices abundant microvilli which extend almost to the surface of the mucus layer covering the epithelium, the thickness of this layer being about $5 \mu\text{m}$. The neighbouring respiratory epithelium consists mainly of non-sensory columnar epithelial cells bearing motile cilia and a few microvilli, with occasional goblet cells interspersed.

Freeze fractured and etched olfactory epithelia showed a pattern of recognisable sensory endings and cilia, and the surfaces of supporting cells. Membrane particles, 8–12 nm across, were present in abundance on the olfactory cilia, at the PF-face (A face) of the membrane (Figs 1–3), and over the whole of the exposed sensory surface of the dendrite. Particles were more numerous on the narrow distal ends of the cilia ($6,000 \mu\text{m}^{-2}$) than on the more proximal ends ($4,300 \mu\text{m}^{-2}$) or on the dendrite ending itself ($3,400 \mu\text{m}^{-2}$); the mean density for the surface of the sensory ending was $5,200 \mu\text{m}^{-2}$.

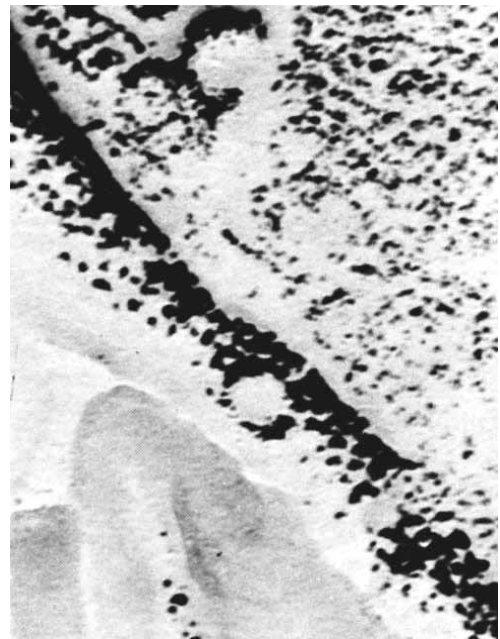


Fig. 3 The tapering distal portion of an olfactory cilium covered in closely packed membrane particles. The ring-like structure in the centre is a surface irregularity ($\times 190,000$).

From measurements made on transmission and scanning electron micrographs it was calculated that each sensory ending had a surface area of at least $160 \mu\text{m}^2$, bearing approximately 10^6 particles. The number of endings per cm^2 of epithelial surface was 5×10^6 . Allowing for the volume occupied by receptor and supporting cell processes, the concentration of particles within the confines of the $5 \mu\text{m}$ layer of mucus at the epithelial surface was estimated to be 10^{19}l^{-1} , which represents a molarity of 2×10^{-3} if each particle is assumed to be a single molecule.

Close to the proximal end of each olfactory cilium, six rows of particles, corresponding to the 'necklace' formation in other cilia¹⁶, could be distinguished. In contrast the cilia of the neighbouring non-sensory respiratory epithelium bore few membrane particles (average 300m^{-2}) although the proximal 'necklace' was present, as described by other authors¹⁷ (Fig. 4). Membrane particles were, however, seen on the short microvilli interspersed among these cilia. Few particles were found at the EF-face (B face) of the membranes of either the olfactory or non-olfactory tissues.

The particles found in the sarcolemma at neuromuscular junctions and in the rod outer segment are similar in packing density to those described here^{6,7}. Membrane particles have been shown in several cases to be proteinaceous¹⁸ and



Fig. 4 The basal region of a non-sensory cilium from the respiratory epithelium. Note the scarcity of membrane particles distal to the necklace ($\times 184,000$).

it can be predicted that the olfactory ciliary membranes are appreciably richer in protein than, for example, those of the motile respiratory cilia. Although the particles could represent ion conductance channels, or some purely structural feature, it seems reasonable to suppose they might also be the receptor sites responsible for binding odorants. Their presence in such high densities may be correlated with the sensitivity of the sensory cells to low concentrations of odorants, and also with the hypothesis that many classes of receptor site, responding to different odorants, may be present on individual sensory cells¹⁹.

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Note added in proof: Similar particles have recently been found in mouse olfactory cilia²⁰.

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Inhibition of PEP-carboxykinase in rat liver by polychlorinated biphenyl

POLYCHLORINATED biphenyls (PCBs) are widely used industrial chemicals which have found their way into the environment, where they have been detected in rainwater, in many species of birds and fish and in human tissues. PCBs accumulate in the body's fat tissues, where they apparently degrade very slowly under natural conditions¹, but recently it has been found² that PCBs may act as carcinogens. The detailed actions of PCBs inside the body, such as their effects on carbohydrate, lipid or protein metabolism, are relatively unknown although they have been shown to alter the liver cell in mouse and monkey³, to affect the immune system⁴ and to alter the activity of microsomal and other enzyme systems⁵⁻⁸. We have studied the *in vivo* effect of PCBs on the activity of two regulatory

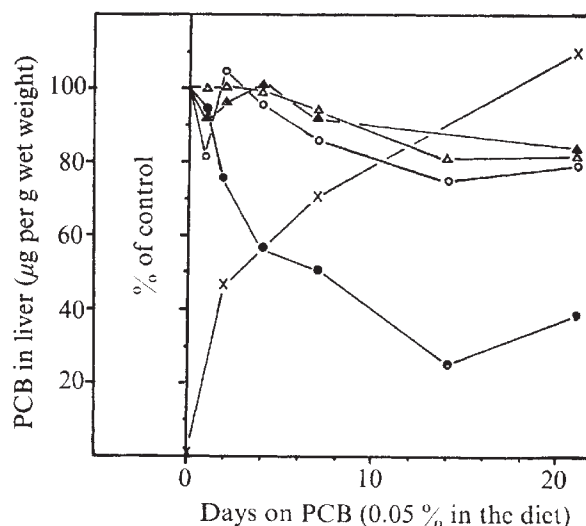


Fig. 1 Effects of PCB (Clophen T64) on PEPCK and FDPase activities in rat liver and on the level of blood glucose. Female rats (Sprague-Dawley, 150-170 g) were used in the experiments. PCB diet was prepared by thoroughly mixing (starmix) 500 g of normal pellets (Eggersmann, Rinteln) with 10 ml of a solution of PCB (Clophen T64) in ethanol giving a final concentration of 0.05% of PCB in the diet. The powder was pasted with water, pellets were formed and dried for 2 d at 37 °C in an oven. The diet for control animals was treated in exactly the same way but without PCB. After the indicated time intervals, livers of 4 rats from each group were removed under ether anaesthesia, homogenised individually in a Potter homogeniser at 0 °C for 1 min using 2 g of liver tissue per 10 ml of 0.25 M sucrose solution. The homogenate was centrifuged for 30 min at 105,000g. PEPCK activity¹¹ and FDPase activity¹² were determined in the supernatant. Blood (0.10 ml) was taken from the aorta descends during removal of the liver, and blood glucose was determined with glucose oxidase¹³. Enzyme activities and content of blood glucose from control animals were set at 100%. ×, PCB in liver; ▲, food intake; Δ, blood glucose; ○, FDPase; ●, PEPCK.

enzymes of gluconeogenesis, phosphoenol-pyruvate carboxykinase (PEPCK) and fructose-1, 6-diphosphatase (FDPase), respectively, in rat liver.

As can be seen from Fig. 1, a diet containing 0.05% PCB (Clophen T64, 60% Cl) diminished the activity of PEPCK by about 50% within a feeding period of 7 d. FDPase activity and the content of blood glucose were also influenced by PCB, but to a much lower extent. Furthermore, the food intake of the animals was not markedly altered by PCB when given in this concentration. During the experimental period the content of PCB in the liver tissue increased from 0.145 (control) to 110 µg per g wet liver. Thus, an *in vivo* content of 70.5 p.p.m. of PCB in the liver lowered the activity of PEPCK to one half of its original activity.

Table 1 Effects of various chlorinated compounds on the activities of PEPCK and FDPase and on the content of blood glucose in rat liver

Dietary addition (0.05%, 7 d)	PEPCK	FDPase	Blood glucose
	(% of control)		
None	100	100	100
Biphenyl	91	101	79
2-Chlorobiphenyl (19% Cl)	119	102	104
Clophen A30 (42% Cl)	66	110	97
Clophen A50 (54% Cl)	53	75	78
Clophen A60 (60% Cl)	51	84	99
Hexachlorobenzene	106	96	100

Rats were fed a normal diet (control), a diet with biphenyl (0.05%) or with various chlorinated compounds (0.05%) for 7 d. Preparation of the diet and experimental details are given in the legend to Fig. 1.

Figure 2 shows the effect of increasing concentrations of PCB in the food after a feeding period of 4 d. A dose of 0.01–0.05% PCB reduced the activity of PEPCK, but did not alter the activity of FDPase, the level of blood glucose and the food intake. Higher concentrations of PCB, however, were obviously toxic because the food intake was reduced and the animals partially fasted. Consequently, PEPCK activity increased in these conditions, since the activity of this enzyme is very sensitive to the animals' nutritional state (see Table 2 and ref. 9). The level of blood glucose and FDPase activity were also lowered by higher concentrations of PCB.

Other chlorinated compounds which are chemically related to PCBs or are concomitants (hexachlorobenzene) were also tested for their *in vivo* ability to influence the activity of PEPCK, FDPase and the level of blood glucose (Table 1). From these compounds PCBs with high chlorine content were most effective on PEPCK, whereas the effect on FDPase activity and on the level of blood glucose varied.

Gluconeogenic conditions such as fasting or feeding a protein diet (poor in carbohydrate) increases PEPCK activity⁹. As is shown in Table 2, after fasting or protein diet PEPCK activity was increased 4.5-fold and threefold, respectively. When PCB was added to the normal diet, the induction rate after

Table 2 PEPCK induction after PCB administration

Treatment of rats	PEPCK (specific activity nmol mg ⁻¹ min ⁻¹)	FDPase	Glucose (mg per 100 ml)
Fed normally	57.8 ± 4.4	34.5 ± 1.5	165 ± 12
Fasted for 16 h (overnight)	260.2 ± 14.8	32.4 ± 1.4	86 ± 3
Clophen T64 (0.05%, 4 d)			
+ fasted for 16 h	121.0 ± 14.3	24.2 ± 2.6	85 ± 12
Protein diet (4 d)	179.6 ± 4.5	27.8 ± 1.2	166 ± 28
Protein diet* with Clophen T64 (0.05%, 4 d)	106.2 ± 5.2	26.7 ± 1.0	121 ± 10

*Protein diet was from Eggersmann, Rinteln. For preparation of PCB-diet and experimental details see legend to Fig. 1.

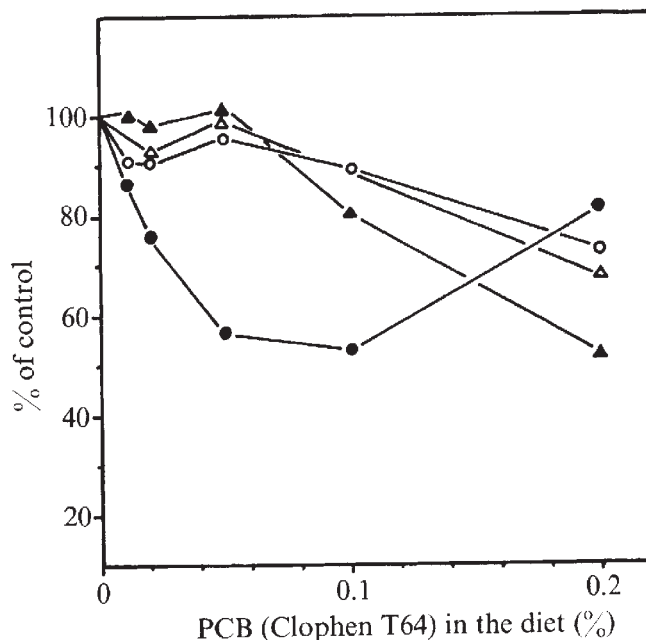


Fig. 2 Effect of increasing PCB concentration on PEPCK and FDPase activities in rat liver. Rats were fed a diet with PCB (Clophen T64) for 4 d. Diet was prepared as described in the legend to Fig. 1, but PCB concentration was varied. Further details see legend to Fig. 1. Symbols as in Fig. 1.

fasting was only twofold and in rats fed a protein diet containing PCB the increase of PEPCK activity was only 1.8-fold. Thus, PCB severely disturbs the ability of the rats to adapt PEPCK activity to gluconeogenic conditions.

Although PCBs are chemically almost inert substances, these compounds nonetheless can alter intermediary metabolism, that is gluconeogenesis. Most determinations described here were carried out between 4 and 7 d on PCB diet (Fig. 2 and Table 1, respectively). This corresponds to an *in vivo* PCB concentration in the liver of about 55–70 µg PCB per g wet liver tissue (see Fig. 1), being in the range of the concentration of physiological substrates. One can suggest, therefore, that PCBs do not act on PEPCK itself but somehow affect its regulation. Since PEPCK activity is closely connected with the level of cyclic AMP and hormones¹⁰, these would be good candidates for further investigations on the mechanism of action of PCBs on intermediary metabolism.

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Two-gene control of T-helper cell induction

THE major histocompatibility complex (MHC) of mouse, man and other species has attracted much attention recently, and that of the mouse is perhaps the most precisely studied mammalian genetic region (reviewed in refs 1-3). Within the space of 0.5 centimorgans are genes coding for transplantation antigens present on essentially all cells (H-2K and H-2D); genes influencing immune responses ("immune response or *Ir* genes"); genes coding for surface alloantigens of restricted tissue distribution (immune response gene region associated antigens or Ia antigens); genes influencing cell interactions, the production of factors and stimulation in the mixed lymphocyte reaction (all in the I region and its subregions I-A, I-B, I-C and so on), as well as genes controlling serum proteins related to complement (S region), and red-cell antigens. Although the products of some of these genetic regions are clear (for example, of the H-2D or H-2K loci) this is not the case for the I region which influences many immune functions, such as delayed hypersensitivity⁴ and antibody production⁵. One group of products of the I region genes constitutes the Ia antigens which have been defined by alloantisera^{6,7}. Other products of these genes are still elusive, and some authors have even suggested that I region gene products are antigen-specific receptors on T cells^{8,9}.

Recently, it was found that the I region influenced the induction of helper cells *in vitro*¹⁰. To induce T-helper cells in our *in vitro* system¹¹, macrophages are required^{12,13}, and with soluble antigens the interaction between T cells and macrophages required genetic similarity in the I-A subregion of the H-2 complex¹⁰. In the mouse, unlike the guinea pig¹⁴ interaction did not depend on direct contact between T cells and macrophages¹², and was mediated by at least two factors¹⁵. (1) A genetically related macrophage factor (GRF) is obtained from antigen-treated macrophages and generates T-helper cells in the absence of macrophages or additional antigen, but only if the macrophage releasing GRF and the T cells are similar at the I-A subregion of the H-2 complex. (2) A nonspecific macrophage factor (NMF), obtained from macrophages incubated without antigen, is not genetically restricted and induces T-helper cells in the absence of macrophages but only with particulate antigens¹⁵. We have characterised the immunochemical nature of GRF and found that it consists of I

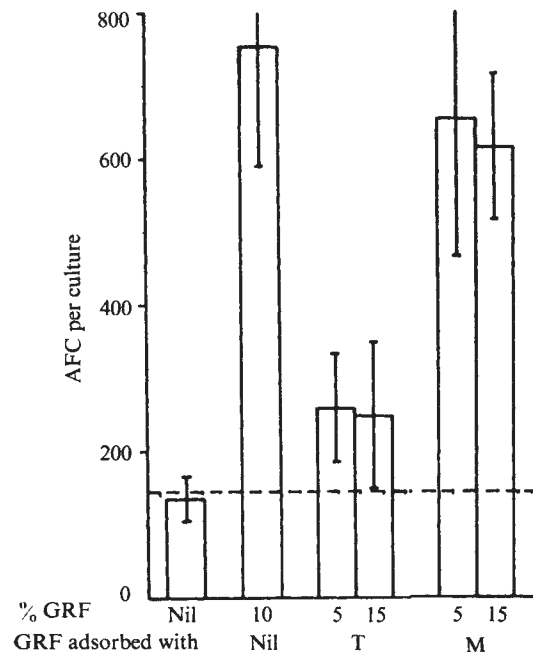


Fig. 1 Evidence for a receptor for GRF on lymphoid cells but not on macrophages. CBA GRF_{KLH} (1 ml) was incubated with either 1×10^8 nylon wool-purified T cells from CBA spleen (T) or with 6×10^7 macrophages (M) (purified by irradiation with 2,000R and adherence to plastic) for 1 h at room temperature. After removal of the cells by centrifugation the adsorbed GRF were added to 15×10^6 nylon wool-purified CBA T cells in a concentration of either 5 or 10% and incubated as described in the legend to Table 1. After 4 d, 2×10^6 living cells from each culture were added to 15×10^6 CBA spleen cells and incubated with TNP-KLH ($0.1 \mu\text{g ml}^{-1}$). Four days later the anti-DNP-response was measured. The dotted line represents the background (15×10^6 spleen cells and TNP-KLH, but no helper cells added). Five experiments of this type have been performed with analogous results. The vertical bars represent the standard error of the arithmetical mean of three independent cultures. To exclude the presence of receptors on B cells, anti- θ -treated and complement-treated spleen cells, cleared of dead cells by low ionic strength buffer²⁹ were used (viability >95%); 10^6 B cells per ml did not remove GRF (for example, in a typical experiment CBAT+GRF: 597 AFC per culture, GRF adsorbed B cells: 480, GRF adsorbed T cells: 47).

Table 1 GRF contains I-A subregion-coded products

CBA GRF _{KLH}	Immunoadsorbents		S/N %	Anti-DNP-response (AFC $\pm$ s.e.)	
				first eluate	acid eluate
—	—	—	—	18 $\pm$ 16	
+	—	—	10	332 $\pm$ 37	
+	A.TL anti-A.AL	(aK ^k)	5	250 $\pm$ 27	30 $\pm$ 17
+	(A.BY $\times$ B10.HTT)F ₁ anti-A.TL	(aI-A ^k)	15	220 $\pm$ 68	0
+	—	—	5	0	363 $\pm$ 46
+	B10.A(4R)anti-B10.A(2R)	(aI-B ^k)	15	0	400 $\pm$ 56
+	—	—	5	199 $\pm$ 26	ND
+	(B10.A(4R) $\times$ 129)F ₁ anti-B10.A(2R)	(aI-C ^{k or d})	15	252 $\pm$ 36	ND
+	—	—	5	423 $\pm$ 112	0
+	—	—	15	373 $\pm$ 23	27 $\pm$ 12

GRF obtained from CBA macrophages by incubating with keyhole limpet haemocyanin (KLH, $10 \mu\text{g}$ per 5×10^6 cells) for 4 d (CBA GRF_{KLH}) were passed over columns containing Sepharose 2B (1 ml of GRF per ml of Sepharose) conjugated with the immunoglobulin fractions of antisera directed against the K^k, I-A^k, I-B^k or I-C^k or ^d subregion of the H-2 complex (first eluate). After washing, the immunoadsorbents were treated with 1 ml of Sørensen's glycine buffer, pH 2.4; the eluates were neutralised immediately and dialysed against phosphate-buffered saline, pH 7.2 (acid eluate). 'First and acid eluates' were tested in different concentration for their helper-cell-inducing capacity by incubating with 15×10^6 nylon wool-purified CBA T cells for 4 d in Marbrook type flasks as described in detail elsewhere^{11,13,15}. Living cells (2×10^6) from each culture (which include helper cells) were then added to 15×10^6 CBA spleen cells and trinitrophenylated KLH (TNP-KLH, $0.1 \mu\text{g ml}^{-1}$), and 4 d later the anti-dinitrophenyl (DNP)-response was measured by the plaque forming ability of the B cells against DNP or TNP (which cross react) coated sheep red blood cells (SRBC). The results are given as arithmetic means of the DNP-AFC (antibody forming cells) $\pm$ s.e. for three independent cultures. Specific AFC are enumerated by subtracting the number of plaques obtained with SRBC from that obtained with DNP-SRBC. The controls include 15×10^6 spleen cells incubated with either TNP-KLH (28 ± 17), or DNP-polyacrylamide beads 3% (1372 ± 175) or no antigen (12 ± 11). (B10.A(4R) $\times$ 129) F₁ anti-B10.A(2R) antiserum (Anti-I-C^{k or d}) was also tested on B10.D2 GRF(H-2^d). It did not adsorb GRF.

region coded products (Ia antigen) complexed with small antigenic fragments with a total molecular weight of 50,000–60,000 (ref. 16). Since Ia antigens are coded for in the I region (which also contains “immune response genes”) we thought that more detailed knowledge about the mechanism of action of GRF might reveal information about helper-cell induction and perhaps how immune response genes work. We have therefore studied the mechanism of binding of GRF to T cells, and report here that at least two genes, located in the I-A subregion, influence helper-cell induction. One of these genes, expressed in macrophages, controls the Ia product of GRF, and the other, expressed on T cells, controls a structure which is the receptor for GRF. This receptor does not react with anti-Ia sera and is thus distinct from conventional Ia antigen.

Though as a result of genetic experiments T cell-macrophage interaction had been mapped in the I-A subregion¹⁰, and GRF, which is one component of this interaction, was serologically shown to be an I region product¹⁶, it was not clear whether GRF was indeed an I-A region product or whether the receptor for GRF was I-A-controlled. Using more specific sera, directed against the H-2K^k, I-A^k, I-B^k and I-C^k regions of the H-2 complex, it was possible to show that the gene(s) responsible for GRF lie in the I-A subregion (Table 1). The evidence for this is that only immunoadsorbents made with anti-I-A^k sera adsorbed out the activity of GRF obtained from CBA macrophages. The adsorbed activity was recovered by acid treatment of the immunoadsorbents, further demonstrating the specificity of the adsorption. An analogous mapping of the genes for GRF in the I-A subregion of other haplotypes (for example, I-A^b or I-A^d) was not possible for lack of specific appropriate anti-I-A region antisera.

We next investigated the interaction of GRF with T cells resulting in the activation of the latter to become specific

helper cells. A simple explanation of this interaction would be binding of GRF by a receptor on helper-cell precursors. To test this, GRF was incubated with either T cells, B cells (10⁶ cells per ml of GRF) or macrophages (6×10⁷ cells per ml) at room temperature (RT) for 1 h. Then, after the cells had been removed by centrifugation, adsorbed GRF was tested for their helper-cell inducing capacity: T cells purified on nylon wool were used. Figure 1 shows that GRF is adsorbed by spleen cells after passage through nylon wool (containing about 1% B cells) as well as by anti- θ serum and complement-treated spleen cells (not shown), but not by macrophages. The latter point is important, making it unlikely that GRF is not synthesised by macrophages. The adsorption by anti- θ -treated spleen cells could be due to (1) residual T cells, (2) dead, but not lysed, T cells or (3) B cells. Indeed, anti- θ -treated spleen cells from which the dead cells were removed before adsorption could not adsorb out GRF activity (for example, see legend to Fig. 1). This indicates that dead cells can remove GRF, presumably because their receptors are still intact. The adsorption by T cells seems to be specific and may be related to the mechanism of GRF, action, for it is dependent on the number of T cells used for adsorption (data not shown), and because T cells incubated with GRF for 1 h at room temperature, then washed thoroughly, and cultured for 4 d were stimulated to become helper cells without further contact with GRF, macrophages or antigen (Fig. 3).

The genetics of adsorption of GRF was investigated. Allogeneic (C57BL/10=B10, H-2^b) spleen or thymus cells did not adsorb CBA GRF at a ratio of 10⁶ cells per ml of GRF in contrast to syngeneic spleen cells or thymocytes (Fig. 2). Even 2×10⁹ allogeneic cells were insufficient. To pursue the genetics of GRF adsorption further, GRF obtained from either CBA, B10.A(4R), AQR, B10 or B10.A(5R) macrophages were first adsorbed with spleen

Table 2 Genetics of the GRF-receptor on spleen cells

T cells from	Ag ($\mu\text{g ml}^{-1}$) or GRF (%)	Helper cell induction		H-2 subregion shared	subregion different	Anti-DNP-response AFC $\pm$ s.e.
		GRF adsorbed with spleen cells from				
B10.BR	KLH 0.1	CBA GRF _{KLH}	10	Nil	—	27 $\pm$ 9
		CBA GRF _{KLH}	5	Nil	—	243 $\pm$ 52
		CBA GRF _{KLH}	15	AQR	I-A ^k , I-B ^k	73 $\pm$ 33
		CBA GRF _{KLH}	15	AQR	I-A ^k , I-B ^k	57 $\pm$ 42
		B10.A(4R)GRF _{KLH}	10	Nil	—	293 $\pm$ 86
		B10.A(4R)GRF _{KLH}	5	CBA	K ^k , I-A ^k	30 $\pm$ 30
		B10.A(4R)GRF _{KLH}	15	CBA	K ^k , I-A ^k	70 $\pm$ 35
		B10.A(4R)GRF _{KLH}	5	AQR	I-A ^k	40 $\pm$ 15
		B10.A(4R)GRF _{KLH}	15	AQR	I-A ^k	3 $\pm$ 3
		AQR GRF _{KLH}	10	Nil	—	240 $\pm$ 30
		AQR GRF _{KLH}	5	B10.A(4R)	I-A ^k	63 $\pm$ 53
		AQR GRF _{KLH}	15	B10.A(4R)	I-A ^k	80 $\pm$ 55
		AQR GRF _{KLH}	15	Nil	—	132 $\pm$ 108
		AQR GRF _{KLH}	15	Nil	—	413 $\pm$ 91
		AQR GRF _{KLH}	15	Nil	—	607 $\pm$ 77
B10	KLH 0.1	B10 GRF _{KLH}	10	Nil	—	570 $\pm$ 73
		B10 GRF _{KLH}	5	B10.A(4R)	I-B ^b , I-C ^b , S ^b , D ^b	670 $\pm$ 35
		B10 GRF _{KLH}	15	B10.A(4R)	I-B ^b , I-C ^b , S ^b , D ^b	467 $\pm$ 61
		B10.A(5R)GRF _{KLH}	10	Nil	—	433 $\pm$ 86
		B10.A(5R)GRF _{KLH}	5	B10.A(4R)	I-B ^b	193 $\pm$ 56
		B10.A(5R)GRF _{KLH}	15	B10.A(4R)	I-B ^b	123 $\pm$ 53
		B10.A(5R)GRF _{KLH}	5	B10	K ^b , I-A ^b , I-B ^b	—
		B10.A(5R)GRF _{KLH}	15	B10	K ^b , I-A ^b , I-B ^b	—
		B10.A(5R)GRF _{KLH}	15	B10	K ^b , I-A ^b , I-B ^b	—
		B10.A(5R)GRF _{KLH}	15	B10	K ^b , I-A ^b , I-B ^b	—

GRF (1ml) obtained from macrophages of various mouse strains were incubated with either A.QR, CBA, B10.A(4R) or B10 spleen cells for 1 h at room temperature. The adsorbed GRF were then tested on their helper-cell-inducing capacity by incubating with either nylon wool-purified B10.BR or B10 T cells for 4 d as described in the legend to Table 1. For each combination, the H-2 subregion (of the k or b haplotype) is listed which is shared by GRF and the spleen cells used for adsorption, and in which they differ, within the left half of the H-2 complex. For example, AQR spleen cells and B10.A(4R) GRF share the I-A^k subregion but differ for the K^k subregion (lines 8 and 9). Thus the diminished activity of the B10.A(4R) GRF after adsorption with A.QR spleen cells indicates that the latter contain GRF receptors on their surface which are coded for by genes of the I-A^k and not of the K^k subregion. Five experiments of this type have been performed, for three of them nylon wool-purified T cells were used for adsorption instead of spleen cells, and other combinations were also tested with analogous results. The controls were:

B10.BR spleen cells + TNP-KLH (0.1 $\mu\text{g ml}^{-1}$)	33 $\pm$ 27
+ DNP-beads 3%	3030 $\pm$ 965
B10 spleen cells + TNP-KLH	107 $\pm$ 12
+ DNP-beads 3%	4470 $\pm$ 1078

Other subdivisions of the H-2I region have been described —I-E and I-J (personal communication from C. S. David and D. B. Murphy). But because these are all to the right of I-B, they do not affect the mapping studies described here.

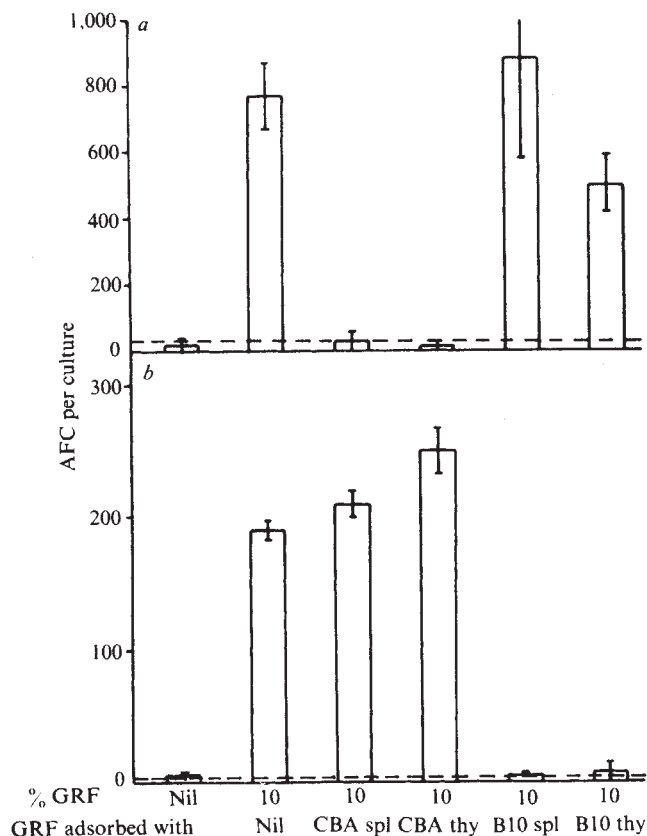


Fig. 2 Evidence for genetic control of the GRF receptor. CBA GRF_{KLH} (a) or B10 GRF_{KLH} (b) (1 ml) were adsorbed with either 1×10^8 CBA or B10 spleen cells (spl) or thymocytes (thy) for 1 h at room temperature. After removal of the cells by centrifugation the adsorbed GRF was tested on their helper-cell-inducing capacity by incubation with 15×10^6 nylon wool-purified CBA (a) or B10 T cells (b) in a concentration of 10% as described in the legend to Table 1. Other concentrations of GRF have also been tested, but for convenience only one concentration is shown here. As controls, unadsorbed GRF or no GRF were used. The dotted line represents the background as described in the legend to Fig. 1. Seven experiments of this type have been performed with analogous results.

cells from AQR, CBA, B10.A(4R) or B10 mice (10^8 cells per ml of GRF) and then tested for their helper-cell inducing capacity with B10.BR and B10 T cells. Table 2 shows that the adsorption of GRF by T cells is also determined by gene(s) of the *H-2* complex, located in the I-A subregion. The simplest interpretation for these results is that the receptor for GRF (which initiates the helper pathway) is controlled by genes in the I-A subregion. These results, however, do not exclude the possibility that the bulk adsorption measured here could be due to a different type of binding unrelated to the manner by which GRF stimulates helper cells. This possibility can be excluded by testing the adsorption of GRF functionally, that is whether adsorbing T cells of different strains were stimulated. Preliminary experiments with B10.A(4R) and AQR mouse strains which are identical only at the I-A_k subregion of the MHC have shown that B10.A(4R) T cells are stimulated by AQR GRF. Together with the other data, this indicates that the T-cell receptor for GRF is involved in the activation of these cells, and that this T-cell receptor is controlled by the I region of the MHC.

Taken together the results indicate that at least two genes, each located in the I-A subregion, but expressed on different cells, regulate helper-cell induction. One gene codes for the Ia part of GRF, the other for the GRF receptor.

It is not likely that macrophages and T cells express both of the genes under consideration, because if so macrophages would adsorb GRF, which they do not (Fig. 1), and T cells would make GRF, which they also do not.

The possibility that the receptor for GRF, since it is controlled by the I region is an Ia antigen was tested by attempting to inhibit the adsorption of GRF by prior treatment of the T cells (which carry these receptors) with anti-Ia sera. A.TH anti-A.TL antisera, with cytotoxic titres of 1/5,000 to 1/1,000 (on spleen) and functional titres of 1/100 or more (on suppressor T cells) did not inhibit binding of GRF to the receptor, even at concentrations of 1/3, whereas the same antisera (at concentrations of 1/3, 1/30 and even of 1/1,000 in other experiments) bound GRF, if incubated together and inhibited its helper-cell-inducing capacity (Fig. 3). These results suggest that the receptor for GRF is not a classical Ia antigen.

It has been shown that the immune response to some antigens is controlled by two genes located in the *H-2* complex. Munro and Taussig¹⁷ postulated a two-gene model for the response to the branched synthetic polypeptide (T,G)-A-L, both mapping in the I-A subregion of the *H-2* complex, with one of these genes expressed in T cells, the other in B cells. The immune response to the terpolymer L-Glu, L-Lys, L-Phe (GLØ) has also been shown to be controlled by two complementary genes in the *H-2* complex, one of which was mapped in the I-A subregion, the other somewhere to the right of the I-C subregion^{18,23}. These reports deal with antigens which are clearly under immune response (*Ir*) gene control, whereas our study was performed with an antigen not known to be under *Ir* gene control. Shevach *et al.*²⁴ have shown in the guinea pig, however, that antisera directed against the guinea pig equivalent of the I region (for example, strain 2 anti-strain-13 sera), only inhibit responses in F_1 (2×13) animals controlled by the relevant I region, for example, the prolifera-

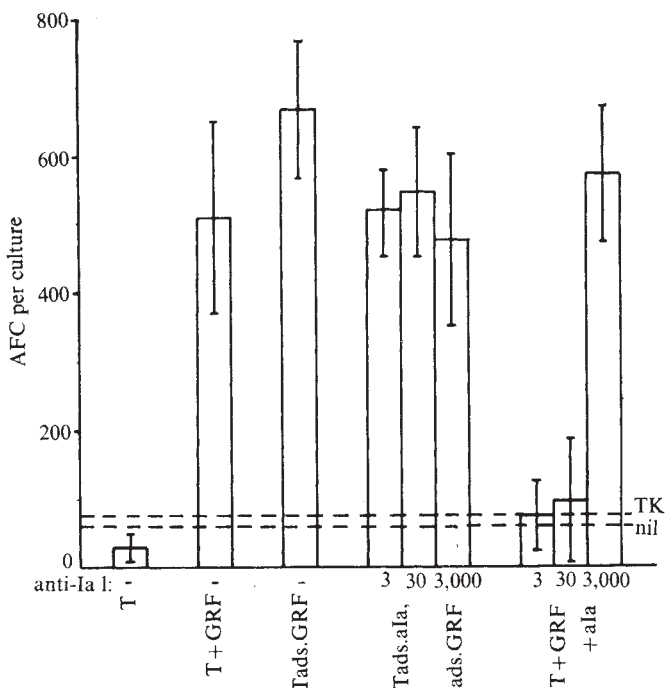


Fig. 3 The GRF receptor on T cells is not Ia antigen. 15×10^6 nylon wool-purified CBA T cells (T) were first incubated with anti-Ia serum (A.TH anti-A.TL batch no. 383) diluted 1:3, 1:30 and 1:3,000 for 3 or 4 h at room temperature. After washing, cells were incubated with CBA GRF_{KLH} (0.1 ml per 15×10^6 cells) for 1 h at room temperature. After repeated washings, the T cells were incubated in Marbrook flasks for 4 d to facilitate helper-cell induction (Tads.ala, ads. GRF). Simultaneously 15×10^6 T cells, GRF (10%) and anti-Ia^k serum in the same dilutions were incubated together for 4 d (T + aIa^k + GRF). As controls, T cells were incubated without GRF (nil), with GRF (T + GRF) or after adsorption with GRF (Tads.GRF). The procedure to measure helper activity is described in the legend to Table 1. The dotted lines represented background levels (spleen cells incubated without antigen (nil) or with TNP-KLH (TK) but without helper cells). Four experiments of this type have been performed with analogous results.

tion response to the synthetic polypeptide GT (a polymer of glutamic acid and tyrosine), an antigen the response to which is controlled by a 13-linked *Ir* gene, was inhibited by anti-13 sera. Schwartz *et al.*²⁵ have obtained similar results in the mouse which imply a functional relationship between *Ir* gene products and Ia antigens. Thus it is possible, even likely, that the reaction described here is a component of the function of H-2-linked *Ir* genes. At present the number of genes influencing helper-cell induction is not known, but results presented here and elsewhere indicate that at least two I region genes are involved.

The most interesting question raised by this study—the mechanism by which GRF is recognised by T cells—is still not resolved. Do helper precursor T cells have two receptors for GRF, one for the Ia product and the other for the antigen, or is there one receptor for “modified Ia” as suggested by Miller’s studies²⁶ in delayed type hypersensitivity and in analogy to the modified H-2 involved in the killing of virus-infected cells²⁷ or of hapten labelled cells²⁸. Our analysis does not permit an answer, but studies are in progress to resolve this issue.

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Liposome-induced morphological differentiation of murine neuroblastoma

LIQUID crystals made of lipids (liposomes), initially devised as models of biological membranes^{1–4}, have more recently

been used as artificial carriers because of their ability to bind and fuse with biological membranes and discharge their soluble content into the cytoplasm^{5–7}. Preliminary studies in our laboratory have shown that liposomes loaded with the nerve growth factor (NGF) induce morphological differentiation in a murine neuroblastoma cell line which does not exhibit morphological changes on addition of NGF to the culture medium. Control experiments with liposomes in absence of NGF however, also resulted in process formation in neuroblastoma (NB) cells. Our attention was therefore focused on the effect of the carrier itself, rather than on the agent carried.

A neuroblastoma cell clone (NB41A₃) was obtained from Dr Augusti-Tocco⁸ and maintained in suspension in F10 medium (Gibco), supplemented with 15% horse serum and 2.5% foetal calf serum (EUROBIO, Paris), penicillin G (200 U ml⁻¹), and streptomycin sulphate (200 µg ml⁻¹) in an humidified incubator conditioned with 5% CO₂ and 95% air, at 37 °C. Egg yolk phosphatidyl choline (PC), phosphatidyl serine (PS), phosphatidyl ethanolamine (PE), phosphatidic acid (PA) and sphingomyelin (SM) were purchased from Lipid Products, South Nutfield, Surrey. N⁶,O²-dibutyryl cyclic AMP, 5-bromodeoxyuridine (BUDR) and papaverine were obtained from Sigma, dimethylsulphoxide (DMSO) was from Merck.

The first set of experiments was directed to find the optimum conditions (liposome composition, time of incubation, temperature) for maximum binding. This process was measured by the amount of ¹⁴C-PC mixed with the phospholipids forming the liposomes, which was not removed by three consecutive washings of the cells after incubation. This measure of ‘tightly bound’ liposomes does not, however, indicate whether binding is followed by fusion and/or interiorisation within the cell cytoplasm. These experiments indicated that a mixture of sonicated PC-PS 10:1.5 (w/w) liposomes progressively attached to, and/or penetrated the cell membrane during a 24-h incubation period at 37 °C. At the end of this time, 0.2–0.4% of the total liposomes present in culture was tightly bound to the cells. Addition of cholesterol to this lipid suspension did not significantly change the pattern of binding. Changing the net charge of the liposome from negative to positive by introducing stearylamine severely reduced the viability of NB cells.

The marked morphological changes (process formation) induced by liposomes are shown in Fig. 1a and b, which compares cultures of the same NB cell line in the absence or presence of this lipid suspension. The fine structure of the liposome-induced processes (Fig. 1c) shows an abundance of microtubules (MT) lying in parallel arrays. In addition to MTs, other fibrillar structures tentatively identified as microfilaments (MF) are also seen in most liposome-treated cultures. The number of cells present in cultures containing liposomes is 40% lower after 24 h and 60% lower after 48 h, than in control cultures. During the same period, the TCA precipitable ³H-methylthymidine is reduced to 30% of controls when expressed as counts per mg of total protein. These findings suggest that liposome-induced morphological differentiation is accompanied by a concomitant inhibition of cell division, although we cannot yet decide whether the two phenomena are correlated or independent. Autoradiographic studies show heavily labelled NB cells following incubation with ¹⁴C liposomes for after only 3 h of incubation (Fig. 2). Whether the radioactivity is confined to the cell surface, or whether it is also within the cytoplasm, is not known.

Several substances have been shown to induce morphological or biochemical maturation of NB cells^{9–13}. In our NB system, however, only dibutyryl cyclic AMP induced process formation, although not to the same extent as liposomes: other agents such as papaverine, 5-bromodeoxyuridine and dimethylsulphoxide, had little or no effect. It is worth mentioning that PS liposomes injected *in vivo*,

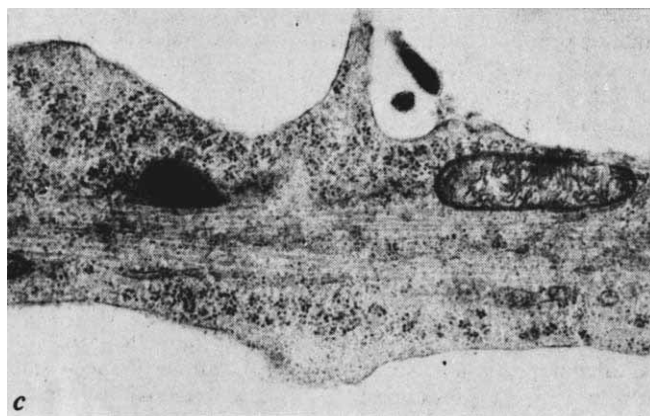
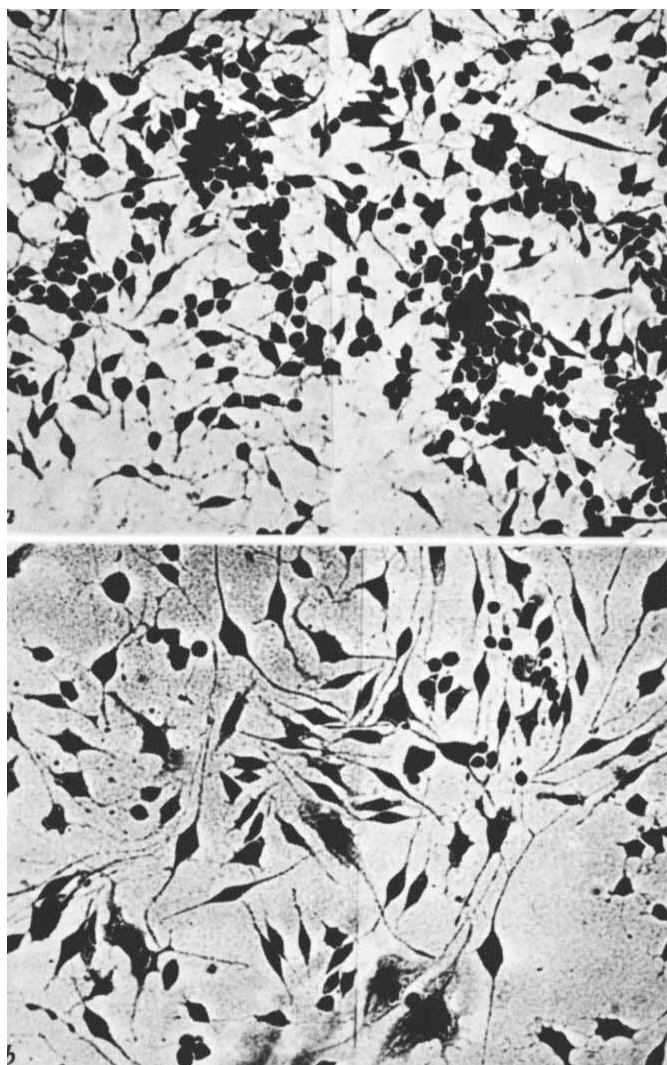
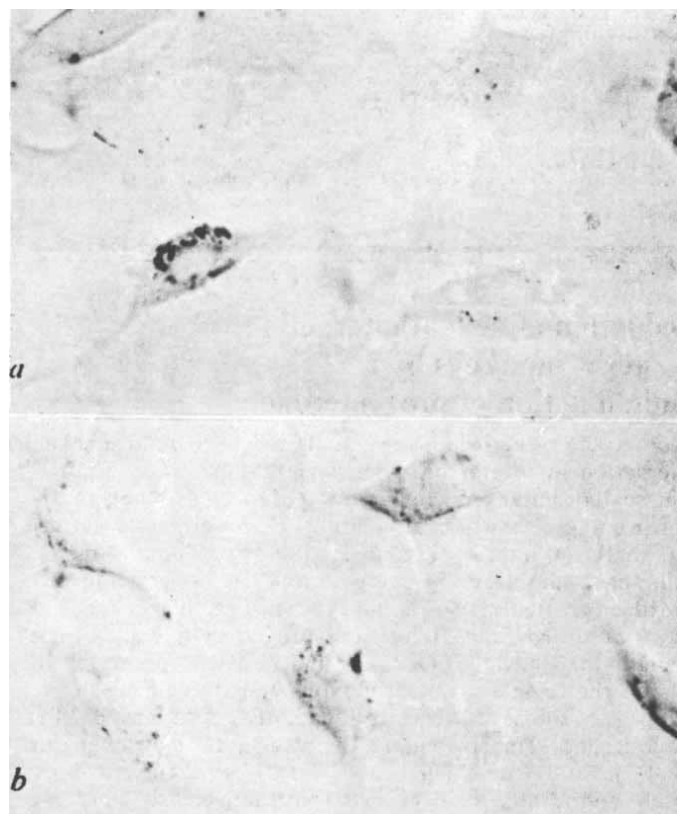


Fig. 1 Effect of liposomes on the morphology of neuroblastoma cells. NB cells (NB41A₃) were plated in a Falcon dish containing coverslips. After 3 h, 100 μ l (2.7 mg of total phospholipids) of the suspension of liposomes was added to a series of cultures (b, c) or the same volume of Gey's balanced salt solution was added to control cultures (a). After 3 d, the cultures were fixed either for light microscope observation (a, b) or for electron microscopic studies (c). a, b, Microphotographs taken using phase contrast were fixed with Duboscq Brasil and stained with haematoxylin. c, Electron micrograph of a portion of neurite from the same culture as shown in (b). Cells were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer with Ca^{2+} added in the same buffer and post-fixed in 1% OsO_4 , dehydrated and embedded in Epon 812. Photo was taken with Philips E.M. 300. Magnifications: a and b, $\times 174$; c $\times 2,079$. Liposomes were prepared as described previously¹⁻³. Generally, 20 mg lecithin from egg yolk was mixed with 3.0 mg phosphatidylserine in 1.0 ml of chloroform-methanol 2:1 (v/v) and dried under a vacuum pump. Gey's balanced salt solution (1 ml) was added and stirred vigorously for 5 min in a vortex mixer. Sonication was in a constant flow of nitrogen gas for 25 min. Liposomes obtained in this way are round, with a diameter of about 120–200 Å measured after ammonium molybdate staining, in the electron microscope.

have been shown to increase brain glucose and decrease catecholamine content of the adrenal medulla^{14,15}.

Our findings raise the question as to the mechanism by which pure lipids, which are normal constituents of the cell membrane, can induce morphological maturation of a neoplastic cell line. Using ^{14}C -PC-loaded liposomes incubated at a concentration of $1.0 \mu\text{mol}^{-1}$ with 4×10^6 cells, we have found that 2–4 nmol of liposomes are bound to, or have penetrated, the cells. This value corresponds to an average of 3×10^5 phospholipid molecules per neuroblastoma cell, that is, (assuming that a sonicated liposome is formed by 2×10^3 phospholipid molecules²), 1.5×10^5 liposomes bound per cell. A liposome bound to the membrane of a living cell may undergo endocytosis, or it may fuse with the membrane itself and become part of its constituents. If the fate of phospholipids is mainly to become constituent parts of the cytoplasmic membrane, their contribution to the

Fig. 2 Autoradiograph of NB41A₃ treated with radioactive liposomes. PC-PS liposomes were prepared as described in Fig. 1 in the presence of $1 \mu\text{Ci ml}^{-1}$ ^{14}C -PC (New England Nuclear, specific activity $1.765 \text{ Ci mmol}^{-1}$ P). After incubation for 3 h, cells (which were grown on coverslips in a Falcon dish) were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer pH 7.2. After 1 h in fixative, cultures were washed in the same buffer, and stained in haematoxylin. The coverslips were air dried and coated with Ilford L4 emulsion. After 50 d of exposure at room temperature, the autoradiographs were developed with Microdol at 21°C for 6 min. Microphotographs were taken with: a, transmission light; b, interference differential (Zeiss-Nomarski) microscope. Magnifications for a and b $\times 824$.



entire surface of each NB cell of diameter $15\ \mu\text{m}$ could be between 5 and 20%, a not insignificant increase for the functional properties of a membrane. Considering that PS molecules are negatively charged and exhibit particular binding capacity for Ca^{2+} and monovalent cations, changes in the net charge of the membrane can be expected following binding and/or fusion of the phospholipids forming the liposomes. The considerable amount of bound phospholipids might also influence the permeability of these cells and effect the organisation of the fibrillar proteins (microtubules, microfilaments) which are essential in axonal growth and elongation.

It remains to be ascertained to what extent *in vitro* process formation in NB cells is a true morphological sign of neural differentiation, since this effect can be induced not only by specific substances¹⁰⁻¹³ but also by noxious agents or treatments^{11,12}. The use of liposomes may contribute to this controversial problem, since their action seems to involve a mechanism which is not dependent on a specific signal, nor do they cause a detrimental effect on the responsive system.

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Reduction of sexual interaction in rhesus monkeys by a vaginal action of progesterone

PROGESTERONE acting in the female seems to reduce sexual interaction in several primate species. It has been reported that sexual activity declines during the luteal phase of the menstrual cycle (when circulating progesterone levels are maximal), in monkeys¹⁻³, lowland gorillas⁴ and humans⁵, and that administering progesterone to ovariectomised, oestrogen-treated rhesus monkeys has a similar effect⁶. A major unresolved question is how progesterone causes these changes in behaviour. One possibility is that progesterone acts on the female's central nervous system, causing her to accept or solicit fewer male mounts. Another is that progesterone somehow alters the vagina thereby changing non-behavioural cues (such as a smell⁷ or tactile qualities) which contribute to her sexual attractiveness. Here we present evidence favouring the second mechanism, since the

reduction in sexual interaction caused by systemic administration of a physiological dose of progesterone to female monkeys could be reproduced by instilling very small amounts of this hormone directly into the vagina.

Ovariectomised female and intact male adult rhesus monkeys (*M. mulatta*) were used. The females were implanted subcutaneously with silastic tubing (Dow Corning, 4 cm long, 3.35 mm inner and 4.65 mm outer diameters) filled with oestradiol-17 β , which results in plasma levels of oestradiol similar to those measured in the follicular phase of the menstrual cycle⁸. Animals were housed separately between observations. Sexual interaction between heterosexual pairs of monkeys selected because they copulated readily without displaying aggression was studied from behind a one-way mirror⁹. In each experiment the same pairs were tested daily in a constant order until the male ejaculated, or for a maximum of 30 min. Here we consider only the following behavioural measures: (1) for the male, mounting rate (number of mounts per min) and the percentage of tests with ejaculation; and (2) for the female, acceptance ratio (the percentage of male mounting attempts accepted) and the number of sexual invitations¹⁰ offered to the male per observation. Plasma progesterone was estimated by radioimmunoassay, using at least two dilutions of plasma¹¹.

In experiment 1 six females were given daily intramuscular injections of 10 mg progesterone in arachis oil, which after 20 d resulted in plasma levels of progesterone (median: $10.74\ \text{ng ml}^{-1}$, range: 5.16-14.52) within the

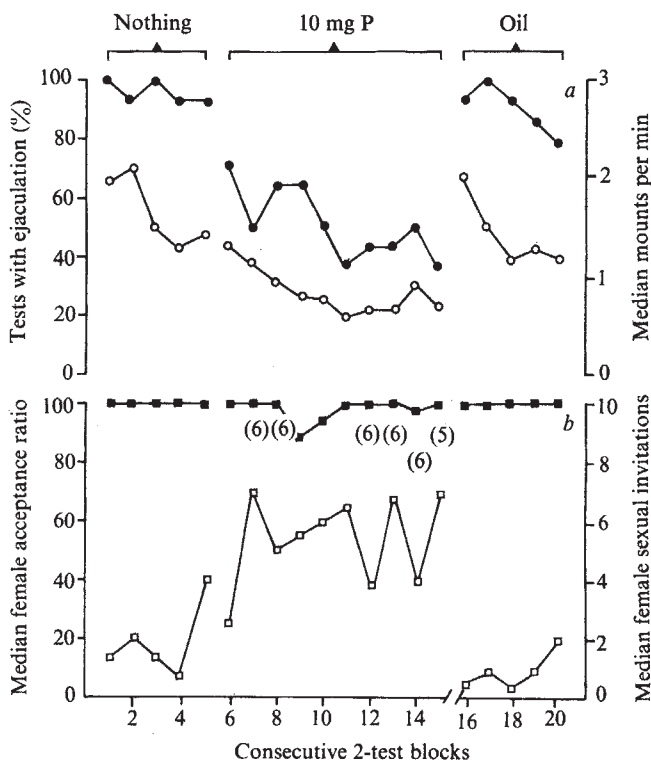


Fig. 1 Effect of $10\ \text{mg d}^{-1}$ progesterone given intramuscularly to the female on sexual interaction in pairs of rhesus monkeys (experiment 1). Five females were tested with one of three males, and another female with two different males. An interval of 32 d, during which no treatment or tests were administered, intervened between test blocks 15 and 16. Two parameters of the males' sexual behaviour are shown in a: $\bullet$, % tests with ejaculation; $\circ$, median mounts min^{-1} ; and two of the females' in b: $\blacksquare$, median acceptance ratio; $\square$, median sexual invitations. Data points are based on seven pairs. Exceptions, which are indicated in parentheses, represent tests in which no acceptance ratio could be calculated for some pairs. The statistical comparisons were made with the initial control condition using 2-tailed Wilcoxon tests, provided an initial Friedman analysis of variance was significant ($P < 0.05$).

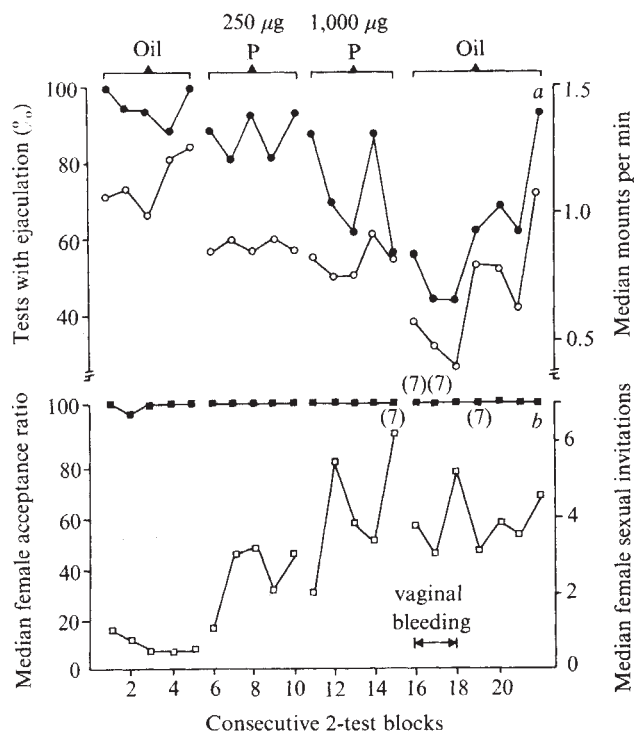


Fig. 2 Effect of low doses of progesterone intravaginally on sexual interaction (experiment 2). Six females were tested with one of six males, and another female with two different males. After each day's behavioural testing, arachis oil solutions were administered by inserting a catheter adaptor attached to a 1-ml syringe into the vagina until it touched the cervix, when it was withdrawn slightly and the injection made. Data points are based on eight pairs, exceptions being indicated in parentheses. Behavioural parameters (a, male; b, female), statistical analysis and symbols as in Fig. 1.

range measured during the luteal phase of the menstrual cycle⁸. This treatment caused significant reductions in the males mounting rate and ejaculation ($P < 0.02$), whereas the females continued to accept virtually all the mounting attempts and actually displayed a threefold increase in sexual invitations ($P < 0.02$) (Fig. 1). Thus, physiological increases in circulating progesterone made the females significantly less attractive to male partners, without altering neural mechanisms so as to depress receptivity. Our results contrast with the reported⁸ reduction in receptivity in some female monkeys which occurred following administration of a pharmacological dose of progesterone (25 mg d^{-1}). In a second experiment, we investigated the possibility that

progesterone affected sexual interaction by acting directly on the vagina.

In experiment 2, instilling progesterone directly into the vagina duplicated the behavioural effects obtained with systemic administration in experiment 1. Figure 2 shows that giving $250 \mu\text{g d}^{-1}$ progesterone intravaginally caused a significant decline in mounting rate and a significant increase in sexual invitations by the females ($P < 0.02$). The incidence of ejaculation declined when the dose of progesterone was raised to $1,000 \mu\text{g d}^{-1}$; however, masculine sexual performance returned to initial control levels after intravaginal progesterone treatment was stopped. Females' acceptance ratios remained high throughout the experiment. Intravaginal administration of $250 \mu\text{g}$ progesterone had no significant effect on plasma levels, although treatment with $1,000 \mu\text{g}$ caused a slight, though significant increase (Table 1A). These findings suggest that progesterone altered sexual interaction primarily by acting on the vagina. The increased display of sexual invitations by females which was observed did not, therefore, result from the direct action of progesterone on neural tissues controlling this behaviour. Instead, it may reflect an attempt by females to compensate behaviourally for a progesterone-induced reduction in sexual attractiveness.

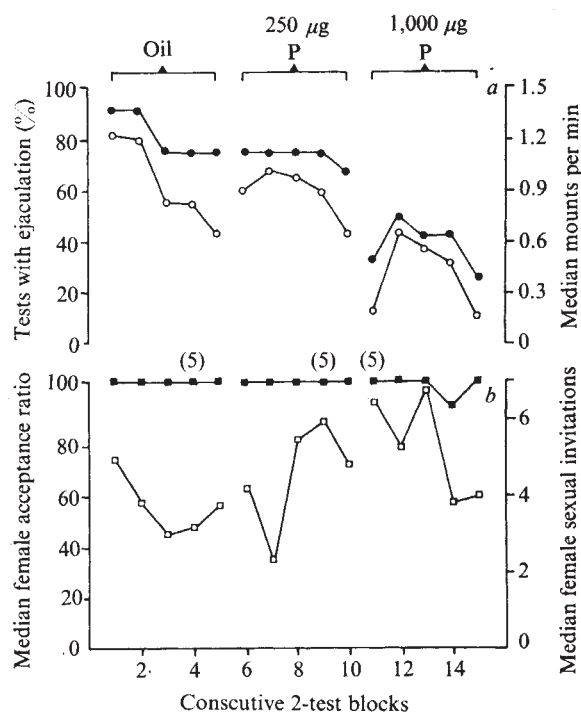


Fig. 3 Effect of low doses of progesterone intramuscularly on sexual interaction (experiment 3). Six females were tested with one of five males. Data points are based on six pairs, exceptions being indicated in parentheses. Behavioural parameters (a, male; b, female), statistical analysis and symbols as in Fig. 1.

These conclusions were supported by the results of experiment 3, in which the same low doses of progesterone were administered intramuscularly instead of intravaginally. Plasma levels of progesterone increased significantly when $250 \mu\text{g d}^{-1}$ progesterone was given intramuscularly (Table 1B); however, this treatment had no significant effect on sexual interaction (Fig. 3). Giving $1,000 \mu\text{g}$ progesterone raised plasma progesterone levels even more and caused a significant decline in males' mounting rate and ejaculation ($P < 0.05$). The females' receptive behaviour was not affected, although in this experiment the increase in their sexual invitations induced by progesterone was not significant. The fact that progesterone in the lower dosage had to be instilled directly into the vagina (experiment 2) to

Table 1 Plasma progesterone in seven ovariectomised, oestradiol-treated female rhesus monkeys following intravaginal and intramuscular administration of progesterone

Site and amount of progesterone administered in 0.1 ml arachis oil		Treatment duration (d)	Plasma progesterone (ng-ml) (Median) (Range)	
A Vagina	0	10	0.12	<0.10-0.41
	250 μg	10	0.29	<0.10-0.43
	1,000 μg	10	0.30*	0.15-0.52
	0	14	0.15	<0.10-0.24
B Thigh muscle	0	10	0.20	<0.10-0.51
	250 μg	10	0.59*	0.28-0.83
	1,000 μg	10	0.81†	0.63-1.32
	0	10	0.12	<0.10-0.41

Within each experiment, comparisons were made with the initial 0 condition using one-tailed Wilcoxon tests. Blood samples were collected 24 h after the last injection in each treatment block.

* $P < 0.05$, † $P < 0.01$.

affect sexual interaction demonstrates that the behavioural effects of this steroid result from its action in this tissue.

Oestradiol enhances sexual attractiveness of female monkeys by affecting the vagina¹². It may act by stimulating the emission of olfactory attractant cues⁷ or by altering the tactile qualities of this tissue. Progesterone could reduce the sexual attractiveness of the females by blocking these effects of oestradiol in the vagina. A comparable anti-oestrogenic action of progesterone has been demonstrated in the oviducts of rhesus monkeys¹³. Alternatively, progesterone could act synergistically with oestradiol to stimulate the production of a vaginal olfactory cue which actively reduces female sexual attractiveness. Either mechanism could account for the reduction in sexual attractiveness which has been reported in different primate species¹⁴ (including humans⁵) at times when the female's plasma progesterone levels are elevated.

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Hypertensive effect of 17 α , 20 α -dihydroxyprogesterone and 17 α -hydroxyprogesterone in the sheep

ANIMAL experimental models of high blood pressure as caused, for example, by renal artery constriction or administration of mineralocorticoid hormones and salt, have yielded basic data on physiological processes which have helped in the diagnosis and treatment of human hypertension. The discovery that ACTH administration causes a reproducible hypertension of rapid onset in the sheep provided a new model for analysis^{1,2}. Certain clinical observations have indicated that there may be as yet unidentified steroids which are causally involved in some hypertensive states: for example, instances of high blood pressure in children and adults which were corrected by dexamethasone treatment^{3,4}, and reversal of high blood pressure in the low renin group of essential hypertensives by inhibitors of steroidogenesis or by spironolactone have been described^{4–6}. We report here the hypertensive effect of 17 α , 20 α -dihydroxyprogesterone

(17 α , 20 α -diOHP; 17 α , 20 α -dihydroxy-4-pregnene-3-one) and 17 α -hydroxyprogesterone (17 α -OHP) in sheep made hypertensive with ACTH. These two progesterones were identified in the adrenal venous effluent from ACTH-hypertensive sheep and were shown to be secreted in greatly increased amounts.

Animal experimental procedures have been described in detail previously^{1,2}. The animals were habituated over several weeks of training to blood pressure measurement by the same observers, and the daily mean was based on 6–8 readings during morning and afternoon. In the absence of a recognisable disturbance in the laboratory, variation in the trained animal during the day was small—less than 5 mmHg. After a control 3–5-d period, intravenous steroid infusions were carried out over 5 d using the same measurement protocol as in the ACTH and the other steroid infusion experiments^{1,2}.

ACTH administration (80 IU d⁻¹) to the intact, but not the adrenalectomised sheep, results in a significant elevation of arterial blood pressure within 24 h and this is sustained over 5–10 d of ACTH treatment¹ (Fig. 1, group *e*). Hypokalaemia, an increase in plasma sodium, and increased water intake and urine output were also observed¹. Changes in blood pressure were not related to changes in external body Na status or in body weight. Intravenous infusion of cortisol, corticosterone, deoxycorticosterone (DOC), aldosterone and 11-deoxycortisol either singly, or in combination at rates to give blood levels similar to those found during ACTH administration (cortisol, 5 mg h⁻¹; corticosterone, 0.5 mg h⁻¹; 11-deoxycortisol, 1 mg h⁻¹; DOC, 25 μ g h⁻¹; aldosterone, 3 μ g h⁻¹), failed to reproduce the elevation in blood pressure² (Fig. 1, group *a*). The combined steroid infusion, however, did reproduce all other metabolic responses seen in the ACTH experiments^{1,2}. In addition, the combined steroids together with 18-hydroxydeoxycorticosterone (18-OHDOC, 100 μ g h⁻¹) did increase blood pressure on the fifth day² (Fig. 1, group *b*). This has been confirmed in an additional six experiments.

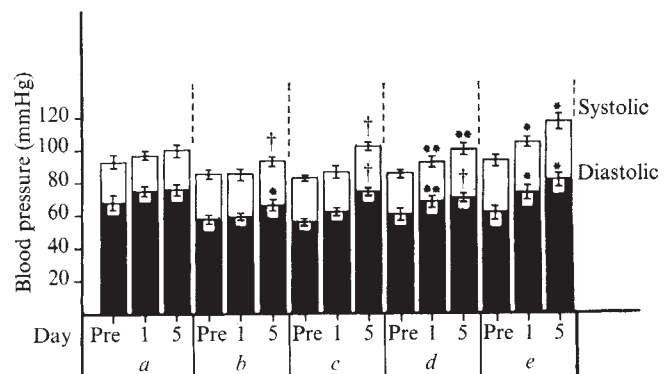


Fig. 1 Mean systolic and diastolic blood pressure ($\pm$ s.e.m.) before (pre) and after 1 and 5 d of the combined steroid infusion (group *a*); combined steroid plus 18-OHDOC (group *b*); combined steroid plus 17 α -OHP (group *c*); combined steroid plus 17 α , 20 α -diOHP (group *d*); and ACTH (group *e*). Statistical comparisons with control (pre) observations have been made using Student's *t* test for paired observations. **P* < 0.05; ***P* < 0.01; †*P* < 0.005.

The animals in the first group (*n* = 5) were given the combined steroid infusion together with 17 α -OHP (Steraloids Inc.) at a rate of 1 mg h⁻¹ for 5 d. This rate approximated the rate of adrenal secretion of the hormone measured during ACTH-induced hypertension in conscious trained sheep with an adrenal transplant⁷, or with an adrenal-jugular bypass operation⁸. The administration rate was thus in the physiological range of adrenal functional capacity to secrete the hormone. Systolic blood pressure increased from 82 $\pm$ 1 (mean $\pm$ s.e.) to 94 $\pm$ 2 and 102 $\pm$ 2 mmHg on the fourth (*P* < 0.05) and fifth day (*P* < 0.005), respectively (Fig. 1, group *d*). Diastolic blood pressure showed a significant increase 56 $\pm$ 1–61 $\pm$ 1 mmHg on

the second day ($P < 0.005$). Neither systolic nor diastolic blood pressure was elevated within the first 24 h.

Animals in the second group ($n = 6$) were given a combined steroid infusion together with $17\alpha,20\alpha$ -diOHP (Steraloids Inc.) at $500 \mu\text{g h}^{-1}$. As in the first group, this rate approximated the physiological output determined during ACTH-induced hypertension and both systolic and diastolic blood pressure were raised significantly within 24 h. Systolic pressure rose from a control of 85 ± 2 to 92 ± 3 mmHg ($P < 0.01$) (Fig. 1, group c); on the final day of infusion, it was 100 ± 3 mmHg ($P < 0.01$). Diastolic blood pressure rose from a control of 60 ± 3 to 68 ± 3 mmHg ($P < 0.01$) and was significantly elevated at 70 ± 2 mmHg on the fifth infusion day ($P < 0.005$).

Preliminary experiments in two animals indicated that neither 17α -OHP nor $17\alpha, 20\alpha$ -diOHP, when infused alone at the same rate as described above, caused any increase in blood pressure over a 5-d period. In a further two experiments where they were given together with the combined steroid infusion, the blood pressure response was similar to that seen in the second group of experiments.

In the study with combined steroid plus $17\alpha,20\alpha$ -diOHP, urinary sodium response involved a rather more sustained initial sodium retention than with ACTH, although blood pressure effects were similar. All other metabolic responses were similar to those in response to ACTH. The more sustained urinary Na retention occurred both with 17α -OHP with combined steroid, and when $17\alpha,20\alpha$ -diOHP and 17α -OHP were infused together with the combined steroid mixture. When either of these steroids was infused alone, without the concurrent combined steroid infusion, they were without effect on urinary sodium excretion.

There is, however, evidence that urinary sodium retention is not the primary mechanism of ACTH hypertension. Sodium depletion of 15–20% of the exchangeable sodium pool by acute parotid duct cannulation before the start of 5 d of ACTH treatment, reduced urinary Na excretion to basal levels. In six animals this did not alter the time course of the blood pressure response to ACTH⁹. Sodium depletion was also carried out in seven animals after the 5-d ACTH treatment, with ACTH being continued over a further 2 d. The blood pressure, though reduced by depletion was still significantly elevated at the end of the sodium depletion period⁹. In five experiments where ACTH was given 24 h after bilateral nephrectomy, there was no difference in blood pressure response from that of intact animals. Although these data support the theory that urinary sodium retention is not the cause of the ACTH hypertension, we cannot formally exclude the possibility that it may be influential in the hypertensive effect of 17α -OHP and $17\alpha,20\alpha$ -diOHP plus combined steroid infusions.

Hypertension associated with excessive endogenous steroid production or administration of exogenous steroids, has been widely reported clinically and in experimental studies. Whereas the cause of increased blood pressure in some of these studies is well known, with others, the cause(s) are not clear. Historically, two classes of steroids—mineralocorticoids such as aldosterone, DOC and corticosterone, and glucocorticoids such as cortisol and 11-deoxycortisol—have been believed to be able to cause hypertension. The exact mechanism by which these types of hypertension are produced and the precise relationship between these steroids, sodium status and blood pressure are still largely undefined. Other naturally occurring steroids—for example, 18-OHDOC and 16- β OH-dehydroepiandrosterone—have been investigated for their hypertensive activities^{4,10}; the former increases blood pressure in the dog¹¹ and sheep² but any hypertensive action of the latter requires confirmation. In the present study, two steroids previously unrecognised as having an hypertensive action were shown to increase blood pressure in sheep when given at high physiological dosages concurrent with, and conditional on, elevated levels of a number of other adrenocortical hormones.

The mechanism by which these two steroids influence blood

pressure is not yet clear. The fact that alone, they do not increase blood pressure, nor do they reduce the salivary Na–K ratio following ipsilateral carotid artery infusion in the Na-deficient adrenalectomised sheep, suggests they are not classical mineralocorticoids. Because doubling the cortisol component of the combined steroid infusion does not reproduce the blood pressure increase of ACTH or addition of $17\alpha,20\alpha$ -diOHP, it is unlikely that they simply add to an existing glucocorticoid-type effect. If they are not glucocorticoids, then recognition of a class of steroids which can increase blood pressure, but which may not act as classical mineralocorticoids, could have important implications for future clinical research and experimental models of hypertension.

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Mode of action of endogenous opiate peptides

AS THE culmination of a search for endogenous ligands of the opiate receptor^{1–4}, the structures of two closely related pentapeptides, isolated from porcine brain, with morphine-like properties in pharmacological tests, have been reported by Hughes *et al.*⁵. The amino acid sequences of the pentapeptides, termed methionine enkephalin and leucine enkephalin, are Tyr-Gly-Gly-Phe-Met (Met⁵-enkephalin) and Tyr-Gly-Gly-Phe-Leu (Leu⁵-enkephalin), respectively. They behave as highly potent opiates in the mouse vas deferens and guinea pig ileum assays⁶. In addition, animals tolerant to morphine are also tolerant to enkephalin⁶. The action of the pentapeptides in the guinea pig ileum⁵ and when injected intracerebrally or intracerebroventricularly^{7–9} is very short lived, perhaps because of proteolytic degradation. A related peptide, with longer chain length has been shown to have a long duration of action¹⁰. Morphine and other narcotics have been shown to bind to the opiate receptor^{11–13} and inhibit adenylate cyclase activity in homogenates of neuroblastoma × glioma hybrid cells^{14,15}, to inhibit cyclic AMP accumulation in intact hybrid cells^{15,16} and in rat brain homogenates¹⁷. We have tested the effects of Met⁵- and Leu⁵-enkephalin on NG108-15 adenylate cyclase activity, and show here that endogenous opiate peptides are potent, receptor-mediated, inhibitors of adenylate cyclase of neuroblastoma × glioma hybrid cells.

NG108-15 hybrid cells were derived (unpublished results of B. Hamprecht, T. Amano and M.N.) by fusion of mouse neuroblastoma clone N18TG-2¹⁸ with rat glioma clone

C6BU-1¹⁹. NG108-15 cells generate action potentials on electrical or chemical stimulation, synthesise acetylcholine (unpublished results of B. Hamprecht, T. Amano and M.N.), and form synapses with striated muscle cells²⁰. They are richly endowed with opiate receptors¹⁴.

The relationship between Met⁵-enkephalin, Leu⁵-enkephalin, and morphine concentrations and basal or prostaglandin E₁ (PGE₁)-stimulated NG108-15 adenylate cyclase activities are shown in Fig. 1a and b, respectively. The concentrations required for half-maximal inhibition of basal adenylate cyclase are 12, 40 and 1,500 nM, respectively, and 20, 120 and 1,500 nM for PGE₁-stimulated activity. Therefore, Met⁵-enkephalin and Leu⁵-enkephalin are approximately 100 and 25 times more potent than morphine as inhibitors of adenylate cyclase activity. The activities of Met⁵- and Leu⁵-enkephalin as inhibitors of adenylate cyclase are approximately equal to their activities as inhibitors of electrically evoked contractions of mouse vas deferens smooth muscle and are five times greater than their activities in a similar assay with guinea pig ileum⁵.

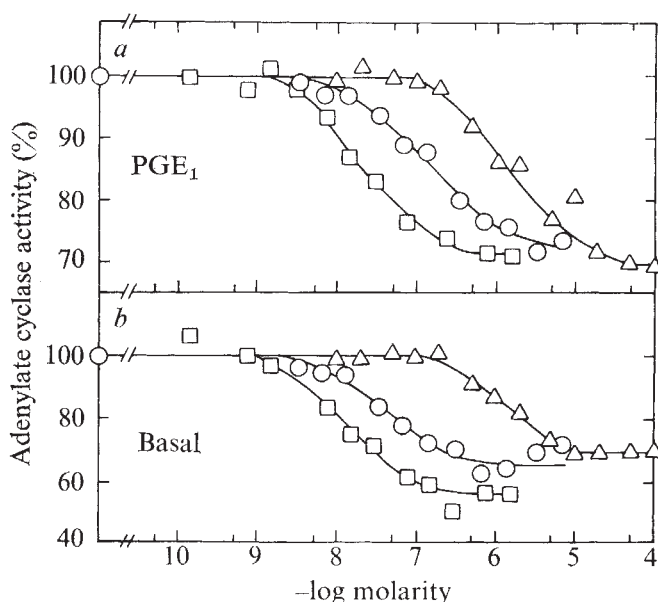


Fig. 1 Basal and PGE₁-stimulated adenylate cyclase activity of NG108-15 homogenates as a function of concentration of Met⁵-enkephalin (□), Leu⁵-enkephalin (○) or morphine (Δ). Reaction mixtures were incubated for 5 min at 37 °C with 97 μg of homogenate protein per tube. Other conditions are described in Table 1. Met⁵-enkephalin was synthesised by the rapid solid-phase procedure of Corley *et al.*²¹. The peptide product (approximately 80% pure) was purified by high-pressure liquid chromatography using a reversed phase system (Corasil, Waters Associates) and an acetonitrile, 0.1-M acetic acid gradient. Leu⁵-enkephalin, a synthetic product, was the gift of Drs R. Simantov and S. H. Snyder.

The effectiveness of Met⁵-enkephalin (1.6×10^{-7} M) as an inhibitor of adenylate cyclase decreases during incubation (Fig. 2), and is lost after approximately 20 min. The degree of inhibition by morphine (2×10^{-5} M) also decreases during incubation, but to a lesser extent. Further work is needed to determine whether Met⁵-enkephalin and morphine are inactivated during incubation or whether an activator of adenylate cyclase is formed.

The effect of naloxone, an opiate antagonist which competitively inhibits narcotic binding to the opiate receptor, on Met⁵-enkephalin or morphine-dependent inhibitions of adenylate cyclase are shown in Fig. 3a and b, respectively. Different concentrations of naloxone were used in the presence of 10^{-8} , 10^{-7} or 10^{-6} M Met⁵-enkephalin or in its absence. Naloxone completely reverses the inhibition of adenylate cyclase by each concentration of Met⁵-enkephalin tested. As expected for competitive interactions at a single

Table 1 Effect of Met⁵-enkephalin and morphine on adenylate cyclase activity in homogenates of neuroblastoma × glioma hybrid and parental cell lines

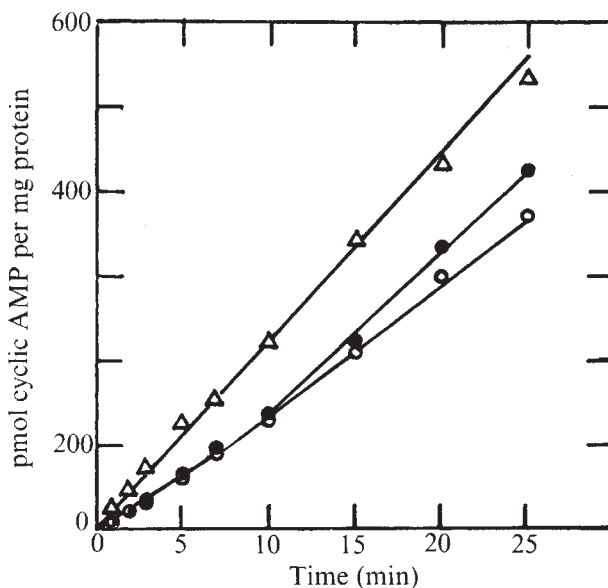
Additions	Cell line		
	Hybrid NG108-15	Neuroblastoma N18TG-2	Glioma C6BU-1
	pmol cyclic AMP per min per mg protein		
None	41.4	13.2	15.8
Met ⁵ -enkephalin	27.2	14.7	15.1
Morphine	35.9	16.8	16.8
PGE ₁	217	115	20.5
PGE ₁ + Met ⁵ -enkephalin	168	120	18.4
PGE ₁ + morphine	164	121	20.2

Adenylate cyclase activity was measured by the procedure of Salomon *et al.*²³ with modifications described before¹⁸. Each reaction mixture contained, in a final volume of 100 μl: 30 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, 160 mM sucrose; 20 mM creatine phosphate; 10 U of creatine kinase; 1 mM ³H-cyclic AMP (10,000 c.p.m.); 0.5 mM RO20-1724 (0.1% ethanol final concentration); 1 mM α-³²P-ATP (10⁶ c.p.m.); and where indicated, 0.16 μM Met⁵-enkephalin; 20 μM morphine sulphate; and 10 μM PGE₁. In addition, 90, 95 and 118 μg of homogenate protein was present for NG108-15, N18TG-2 and C6BU-1, respectively. Tubes were incubated for 3 min at 37 °C. Reactions were stopped with 1 ml of 5% trichloroacetic acid containing 1 mM ATP and 1 mM cyclic AMP. ³²P-cyclic AMP and ¹⁴C-cyclic AMP (for recovery) were counted after purification as described by Salomon *et al.*²³. Cell lines, growth conditions and the adenylate cyclase assay method have been described previously (refs 14 and 15 and unpublished results of B. Hamprecht, T. Amans and M. N.). Washed cells, suspended in 0.32 M sucrose, 0.01 M Tris-HCl at pH 7.5 were stored at -80 °C before use.

site, the amount of naloxone required to reverse the actions of Met⁵-enkephalin or morphine is a function of the affinity of the receptor for the ligands and their concentrations. The dissociation constant of naloxone, K_d , calculated²² from the data in Fig. 3a and b is 3×10^{-8} M for reversal of Met⁵-enkephalin inhibition and 2×10^{-8} M for reversal of morphine inhibition (see legend to Fig. 3). These values agree well with the dissociation constant of naloxone of 2×10^{-8} M previously determined by direct measurement of the binding of ³H-naloxone to the opiate receptor of the hybrid cells¹⁵.

As Table 1 shows, Met⁵-enkephalin and morphine inhibit adenylate cyclase in homogenates prepared from NG108-15 hybrid cells, but not adenylate cyclase of the glioma parent, C6BU-1, which lacks narcotic receptors, or the neuroblastoma parent, N18TG2, which has fewer narcotic

Fig. 2 Time course of cyclic AMP formation by NG108-15 homogenates (97 μg per protein per tube) in the presence of 2×10^{-5} M morphine (○), 1.6×10^{-7} M Met⁵-enkephalin (●), or in the absence of added inhibitors (Δ). Assay conditions were as for Table 1.



receptors than NG108-15 hybrid cells^{14,15}. These data, together with the naloxone reversal of enkephalin inhibition, show that both opiate receptors and enkephalin are required for the inhibition of adenylate cyclase activity.

The apparent inhibition constants for adenylate cyclase of Met⁵- and Leu⁵-enkephalins as well as morphine and some peptides which are fragments of enkephalin are summarised in Table 2. The K_i for Met⁵-enkephalin is 12–20 nM. Leucine enkephalin is three to six times less active an inhibitor than Met⁵-enkephalin and the enkephalin fragments tested had little or no effect on adenylate cyclase activity. In other experiments, not shown here, the di- and tripeptides did not antagonise morphine-dependent inhibition of adenylate cyclase. Met⁵- and Leu⁵-enkephalins are somewhat more potent inhibitors of basal, than PGE₁-stimulated adenylate cyclase. Morphine usually behaves similarly, although not in the experiment shown here. These observations show that opiate and PGE₁ receptors are functionally coupled either by allosteric interactions or by competition for the same site on the enzyme.

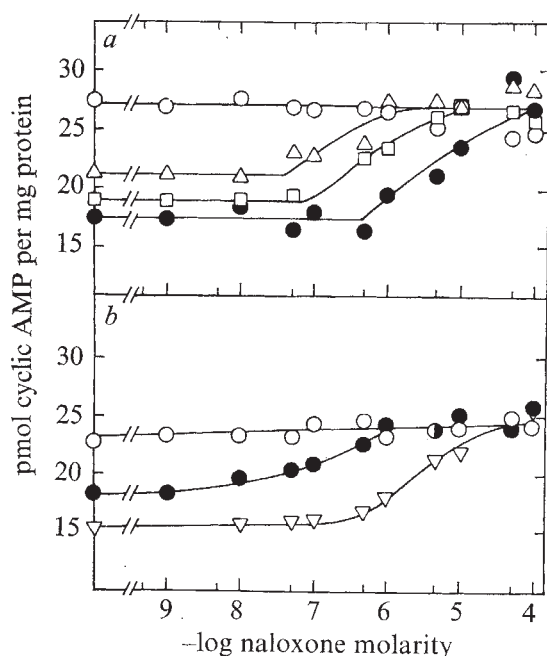


Fig. 3 *a*, relationship between naloxone concentration and the reversal of adenylate cyclase inhibition by 0.01 (△), 0.1 (□), or 1 μM (●) Met⁵-enkephalin. Also shown is the relationship between naloxone concentration and adenylate cyclase in the absence of Met⁵-enkephalin (○). Assays were for 5 min with 87 μg of homogenate protein per tube. *b*, a similar experiment without morphine (○), or with 1 μM morphine (●); or 100 μM morphine (▽). Reaction mixtures (90 μg of homogenate protein per tube) were incubated for 5 min. Other assay conditions are described in the legend of Table 1. Equilibrium binding constants (K_d) for naloxone were calculated by finding the concentration of naloxone at which the adenylate cyclase activity, in the presence of the highest concentration of inhibitor, is equal to that measured in the absence of naloxone at an inhibitor concentration 100 times lower. This concentration of naloxone is, by the dose-ratio method²³, equal to 99 times K_d .

Our experiments show that endogenous opiate peptides can be assayed rapidly by determining the opiate-peptide-dependent inhibition of adenylate cyclase which is reversed by naloxone. The range of the assay is 0.2–5.0 pmol of Met⁵ enkephalin per 100 μl of reaction mixture or 0.1–2.5 pmol per 50 μl of reaction mixture. Thus, we have found, in collaboration with Goldstein and Cox, that a peptide which has been purified extensively from pituitary tissue²⁴ and has a higher molecular weight than enkephalin is a potent inhibitor of both basal and PGE₁-stimulated adenylate cyclase of NG108-15 cells. Enkephalin antagonists can be assayed in a similar manner.

Table 2 Activity of peptides and morphine as inhibitors of adenylate cyclase in NG108-15 homogenates

Compound	K_i^* (nM)	
	Basal	PGE ₁
Tyr-Gly-Gly-Phe-Met (Met ⁵ -enkephalin)	12	20
Tyr-Gly-Gly-Phe-Leu (Leu ⁵ -enkephalin)	40	120
Morphine	1,500	1,500
Tyr-Gly-Gly	> 1,000,000†	—
Gly-Gly-Phe	> 1,000,000‡	—
Phe-Leu	> 1,000,000‡	—

Adenylate cyclase activity was determined as described in the legend of Table 1.

*Concentration of peptide required for half-maximal inhibition of basal or PGE₁ (10⁻⁵ M)-stimulated adenylate cyclase. Maximal inhibition of adenylate cyclase activity usually is 30–60% of total activity¹⁵.

†Slight activity at 10⁻³ M (0–20% inhibition of adenylate cyclase).

‡No inhibition at 10⁻³ M.

An important question, that can be studied with cultures of NG108-15 cells, is whether the endogenous opiate peptides are addictive. As reported previously^{25,26}, NG108-15 cells and perhaps animals as well²⁷ chronically exposed to morphine, become tolerant to and dependent on morphine due to its dual action in inhibiting adenylate cyclase and eliciting a gradual increase in adenylate cyclase activity which compensates for the inhibition. Experiments are in progress to test whether hybrid cells become tolerant to and dependent on enkephalin and other opiate-like peptides.

Opiate peptides, 5–31 amino acid residues long^{28–30}, as well as β -melanocyte-stimulating hormone (β -MSH) apparently are derived by proteolysis of a common precursor, β -lipotropin³¹, which had been isolated from pituitary³². Thus, β -lipotropin is a precursor of both an activator of adenylate cyclase (β -MSH)³³, and inhibitors of this enzyme (opiate peptides). Presumably, β -MSH and the endogenous opiates are recognised by different species of receptor which may be on the same or on different cells. Thus, positive and negative responses could result in separate cells or a single cell from different peptides derived from the same precursor.

The endogenous opiate peptides seem to be neurotransmitters or hormones which are destined for neurones with opiate receptors. The opiate peptide-receptor complex is a potent inhibitor of adenylate cyclase and thus the activations of adenylate cyclase by other species of neurotransmitters and hormones are suppressed. In effect, the opiate peptides act as pleiotropic desensitisers of many kinds of receptors which, in concert with the corresponding ligands, activate adenylate cyclase. Prolonged exposure to the opiate peptides may lead to an increase in adenylate cyclase activity as previously observed with morphine and other narcotics^{25,26}. Thus, the opiate peptides may regulate the perception of incoming messages by neurones with opiate receptors in both a negative and positive manner.

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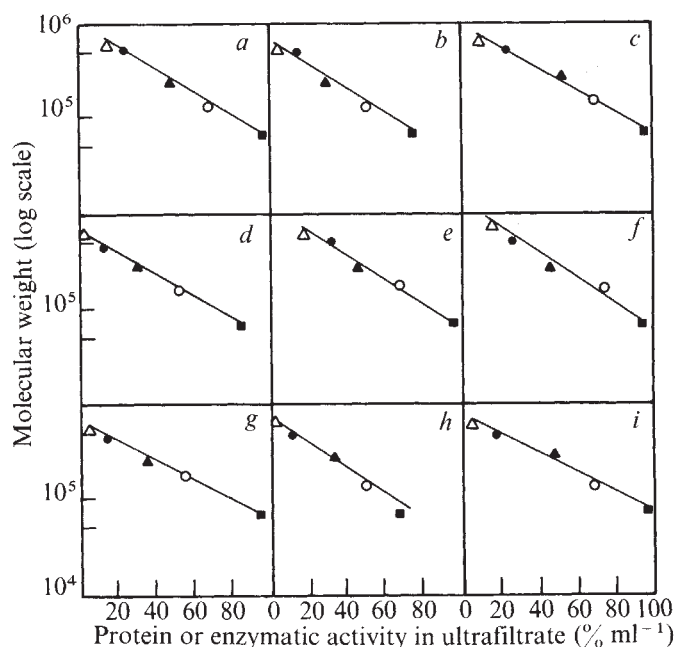


Fig. 1 Linear relationships between percentage marker proteins in filtrate and molecular weight on 9 different XM100 membranes labelled A-I. Each point represents the mean of at least 10 determinations. ■, Albumin; ○, gamma globulin; ▲, β -glucuronidase; ●, urease; △, β -galactosidase.

Molecular weights of antihemophilic factor and von Willebrand factor proteins in human plasma

HUMAN factor VIII contains at least two biological activities, antihemophilic factor (AHF) and von Willebrand factor (VWF). When highly purified, factor VIII is estimated to have a molecular weight of over 1.12×10^6 , as determined by gel chromatography or sedimentation equilibrium centrifugation¹⁻⁴. Some investigators²⁻⁴ consider factor VIII a single glycoprotein with a number of covalently linked subunits. Since factor VIII can be dissociated in certain conditions, others⁵⁻⁷ consider it to be a two-molecule complex consisting of a multi-subunit high molecular weight protein ($> 10^6$) with VWF activity that acts as a carrier molecule for a lower molecular weight (2.4×10^5) subunit with AHF activity. A third model suggests that both AHF and VWF are high molecular weight, separate proteins consisting of disulphide linked subunits^{8,9}.

These interpretations do not adequately explain certain clinical and laboratory observations. For example, von Willebrand patients infused with factor VIII preparations show a secondary rise in AHF activity when no measurable VWF activity or factor VIII-related antigen is present¹⁰. The molecular weight of the AHF proteins in this secondary rise, as determined by Agarose gel chromatography, showed that some AHF activity was associated with proteins with a molecular weight of at least 1×10^6 and some with proteins of considerably lower molecular weight; but neither protein showed VWF activity¹¹. Furthermore, low molecular weight AHF has been found to aggregate with itself⁸. These findings could be interpreted more readily if native AHF and VWF proved to be two different molecules and the circulating AHF and VWF were low in molecular weight, comparable to that reported for the subunit size of reduced factor VIII.

Using rapid quantitative ultrafiltration of whole plasma to estimate the molecular weight of AHF and VWF and study their interactions, we determined that each native protein has a molecular weight of less than 300,000 and they exist in plasma as separate molecules.

Whole blood from healthy male and female adults was collected in 3.8% citrate (9:1). All subsequent processing steps were carried out at the specified experimental temperature.

Plasma low in platelets was prepared by two centrifugations for 5 min at 16,000g and used immediately.

New Amicon 25-mm diameter XM100 ultrafiltration membranes were routinely cleaned by ultrasonication (Millipore Ultrasonic Cleaner Model #XX66 008 00) for 60 min in distilled water before use and by sonication for 15 min in distilled water before each application of proteins. Each filter was calibrated by ultrafiltration of several enzymes and other protein markers of known molecular weight and recalibrated before each subsequent use. The same filter could be used many times and between uses was stored at 4 °C in distilled water. Other methods for preparing and cleaning the membranes, including those suggested by the manufacturer, proved unsatisfactory and experiments in which they were used were, in general, not reproducible.

Solutions of each protein molecular weight marker containing 1.0 mg ml⁻¹ (100%) were individually ultrafiltered and the

Table 1 Apparent molecular weight of native AHF and VWF determined by ultrafiltration at 37 °C on ultrasonicated Amicon XM100 membranes

Experiment	Molecular weight	
	AHF	VWF
1	220,000	—
2	210,000	—
3	220,000	—
4	230,000	—
5	240,000	—
6	240,000	260,000
7	240,000	260,000
8	235,000	280,000
9	250,000	290,000
10	240,000	—
11	240,000	—
12	240,000	280,000
13	240,000	270,000
14	230,000	280,000
15	260,000	300,000
16	240,000	300,000
Mean $\pm$ s.d.:	236,000 $\pm$ 12,000	280,000 $\pm$ 15,000

The difference between the mean molecular weights calculated from the paired data is highly significant ($P < 0.001$).

filtrate was assayed for enzyme activity or protein concentration. The results were expressed as the percentage of the initial concentration per ml. The resulting calibration curves (Fig. 1) show a linear relationship between the percentage of protein recovered in the ultrafiltrate and the molecular weight. Each point represents the mean of 10 determinations on one of 9 different XM100 membranes. The precalibrated ultrasonicated membranes could thus be used to determine the molecular weights of various proteins from 68,000 to ~600,000, without loss of biological activity.

The first ml of ultrafiltrate was collected from 3.0 ml of fresh plasma filtered at 37 °C under nitrogen (10 pound/inch²). The filtrate and retentate were then examined for AHF activity (two-stage assay)¹², ristocetin VWF activity^{13,14}, and factor

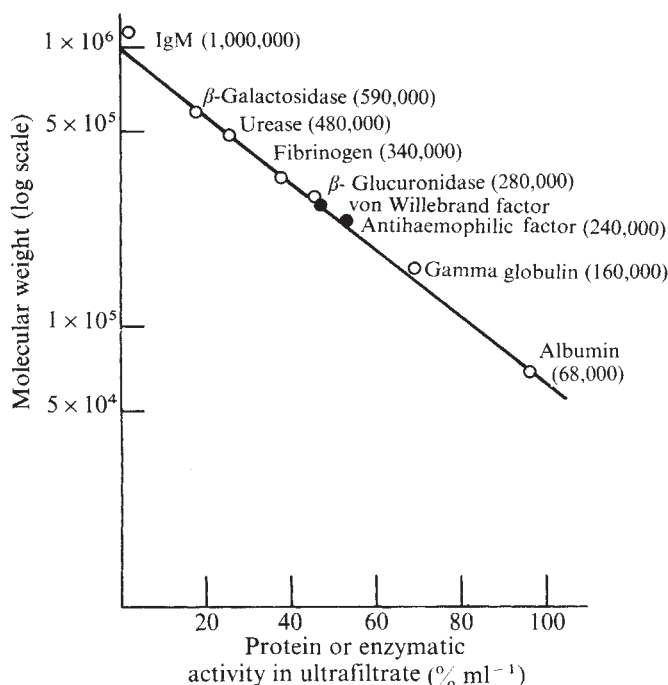


Fig. 2 Molecular weight of AHF and VWF determined from the percentage of fresh plasma in the ultrafiltrate in a single typical experiment using membrane E. Marker proteins and plasma were filtered through an XM100 membrane at 37 °C under nitrogen (10 pound/inch²).

VIII-related antigen (radioimmunoassay)¹⁵. Between the filtrate, the retentate and the material trapped on the filter support, virtually all the AHF or VWF activity in the sample could be accounted for.

In these conditions, 53% of AHF and 47% of VWF and antigen activity were recovered in the ultrafiltrate in a typical experiment. As evident from the calibration curve, the percentage of AHF or VWF in the ultrafiltrate corresponded to a molecular weight of 240,000 for AHF and 270,000 for VWF (Fig. 2). With use of a number of different calibrated membranes, a mean molecular weight of $236,000 \pm 12,000$ for AHF and $280,000 \pm 15,000$ for VWF was determined (Table 1).

The fact that the AHF and VWF in the 1 ml of filtrate was not a selected portion of the proteins in the 3 ml of sample applied was demonstrated when all 3 ml of plasma were filtered through a 43 mm instead of a 25 mm diameter membrane and similar molecular weight values were obtained.

To assess the effect of prolonged incubation on the filterability (molecular weight) of AHF and VWF, fresh plasma was incubated at 37 °C, and samples were assayed periodically. The proteolytic inhibitors Trasylol (50 kI Units ml⁻¹) and benzamidine (2×10^{-3} M) were added to prevent degradation of proteins during the experiment. After 6 h of incubation, the apparent molecular weight of AHF increased from 240,000 to 380,000 whereas the apparent molecular weight of VWF increased from 280,000 to more than 600,000. This increase was

attributed to aggregation of the AHF and VWF *in vitro*. The rate of aggregation proved to be faster at lower temperatures (25 or 4 °C).

Since the rate of aggregation appeared to increase with time and at low temperature, it was not surprising that the ultrafiltrates of cryoprecipitate and two commercial factor VIII preparations contained no measurable AHF or VWF activity, both of which were found in the retentate.

In summary, we have shown that the molecular weights of AHF and VWF in fresh human plasma are ~240,000 and 280,000. Their different molecular weights and rates of aggregation suggest that they are separate proteins, a conclusion consistent with other data from our laboratory using solid-phase polyelectrolyte fractionation. High molecular weight factor VIII seems to be an artefact arising from blood processing, low temperature storage, or fractionation procedures.

Quantitative ultrafiltration offers an important new method for the rapid, non-destructive estimation of the molecular weight of microgram quantities of proteins.

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N-terminal amino acid sequences of variant-specific surface antigens from *Trypanosoma brucei*

THE major pathogenic African (salivarian) trypanosomes can evade the immune response through a process of antigenic variation (reviewed in ref. 1). Mainly due to Gray's work^{2,3}, this variation is thought to be phenotypic—involving sequential expression of alternative genes and not resulting from contemporaneous mutation. The capacity for variation which exists within a clone, isolate or species, or within strains circulating within a geographical area, is uncertain (though probably extensive) and is relevant to an evaluation of the feasibility of developing vaccines. Although serotyping of trypanosome populations has been refined through the use of fluorescence methods^{4,5}, there is a need to characterise the relevant antigens and investigate the molecular basis of antigenic variation. Other workers⁶⁻⁹ identified putative variable antigens and found them to be soluble glycoproteins¹⁰⁻¹², probably located in the surface coat^{13,14}. The antigen preparations obtained, however, were generally heterogeneous, frequently unstable or in low yield. A recent report¹⁵ described the complete purification in good yield of individual variant-specific surface antigens (VSSAs) from a series of clones of *Trypanosoma brucei*. The VSSAs seem to be the primary

Variants

	1	10	20	30
I	Thr-Asn-Asn-His-Gly-Leu-Lys-Leu-Gln-Lys-Ala-Glu-Ala-Ile-Cys-Lys-Met-Cys-Lys-Glu-			
II	Ala-Lys-Glu-Ala-Leu-Glu-Tyr-Lys-Thr-Trp-Thr-Asn-His-Cys-Gly-Leu-Ala-Ala-Thr-Leu-Arg-Lys-Val-Ala-Thr-Gly-Val-Leu-Thr-Lys-Leu-Lys-Ser-His-Ile-			
III	Thr-Asp-Lys-Gly-Ala-Ile-Lys-Phe-Glu-Thr-Trp-Glu-Pro-Leu-Gln-Leu-Leu-Thr-Gln-Asp-Phe-Gly-Asn-Leu-Tyr-Asn-Lys-Ala-Lys-Lys-Asn-Leu-Asp-			
IV	Ala-Glu-Ala-Lys-Ser-Asp-Thr-Ala-Ser-Gly-Ser-Val-Asn-Ser-Pro-Gln-Thr-Glu-Ala-Thr-Tyr-Ala-Gln-Leu-Ala-Lys-Thr-Leu-Gln-Arg-Ala-Leu-Asp-			

Fig. 1 Amino terminal sequences of variant-specific surface antigens from four successive trypanosome variants. Sequences were obtained by automated liquid-phase and solid-phase techniques (see text). Phenylthiohydantoin derivatives were identified by high-pressure liquid chromatography on a column of microbondapak-C₁₈ reverse-phase support (Waters Associates) using a Waters liquid chromatograph fitted with an ultraviolet detector. Solvent 1 was water-methanol-acetic acid (90:10:0.25), pH 4.1, and solvent 2 was water-methanol-acetic acid (10:90:0.025). A linear gradient of 95% solvent 1:5% solvent 2 to 40% solvent 1:60% solvent 2 was run at a flow rate of 2 ml min⁻¹. Normal sample quantity was 1–5 nmol, but where less sample was available, acetone was added to solvent 1 (50 µl l⁻¹) to give a flat baseline at high detector sensitivity. Identifications were confirmed by either thin-layer chromatography (all residues except His and Arg for positions 1–25), gas-liquid chromatography (non-polar residues) or amino-acid analysis after back-hydrolysis with HI (all residues except Trp and Met) (for details see ref. 19). Average repetitive yield was 93.5%. Residues in italics are interim assignments.

mediators of antigenic variation in *T. brucei*. Each antigenically homogeneous trypanosome clone yields one predominant glycoprotein antigen composed of a single polypeptide chain (apparent molecular weight 65,000 as determined by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis) and, depending on the variant, 7–17% (w/w) carbohydrate (unpublished results of J. G. Johnson). The VSSA seems to be the major or sole constituent of the surface coat and comprises 5–10% of the total cell protein. Studies of the amino acid sequences, carbohydrate constitution and cell-surface organisation of several VSSAs are in progress, and we report here the N-terminal amino acid sequences of VSSAs isolated from four closely related variants.

Antigens were purified as described previously¹⁵, from a series of clones prepared from cell populations isolated during 3 weeks from a single rabbit infected with a cloned strain (427) of *T. brucei*. N-terminal sequences (Fig. 1) were generally determined on approximately 8-mg amounts of intact S-¹⁴C-carboxymethylated antigens using a dilute Quadrol programme¹⁶ in a Beckmann 890C sequencer. Runs were performed in duplicate. The sequence of variant II was identical in two samples purified on separate occasions. Limited tryptic digestion (unpublished results of G. A. M. C. and J. G. Johnson) of variants I and III produced in each case a single large fragment of molecular weight 48,000 and 45,000, respectively (estimated from SDS-polyacrylamide gel electrophoresis). After purification on Ultrogel AcA 54 (variant III) or by isoelectric focusing (variant I), 1 mg of each tryptic fragment was attached to 3-aminopropyl glass using phenylene diisothiocyanate¹⁷ and was degraded in an LKB 4020 solid-phase sequencer using a single cleavage programme (LKB Biochrom Ltd) similar to that described by Laursen¹⁸. The N-terminal sequences of these fragments from variants I and III were identical to the respective intact proteins through residues 2–15. The results for variants I and II extend and confirm those obtained previously using a micro solid-phase technique¹⁷.

These results confirm the homogeneity of the purified antigens and demonstrate an absence of sequence homology in the N-terminal region. Sequential cleavage techniques generally permit identification of the initial 30–40 residues of an intact protein. This represents only 6% of the VSSA molecule. Future work, involving subfractionation of the antigen, will extend these sequences and may reveal homologies in other regions of the molecules. Other evidence (unpublished results of G. A. M. C. and J. G. Johnson) however, suggests that extensive variation occurs along most of the polypeptide chain, but that nevertheless there may be some conservation of tertiary structure, as indicated by the characteristics of selective tryptic cleavage of the native antigens. The isolation of large tryptic fragments with intact N-terminal regions containing several lysine residues from variants I and III suggests that the N-

terminus of the native VSSA is relatively protected from proteolytic attack. This observation, coupled with the reproducibility of sequences obtained with different preparations of VSSA, makes it likely that the reported sequences represent the true N termini of the intact antigens.

Except in respect of their lack of homology, the reported sequences are without intrinsically striking features. The amino acids are present in proportions which reflect the overall VSSA amino acid compositions. Even if the amino acid sequences of the VSSAs analysed in the study reported here are later shown to be atypically hypervariable, it seems that evolution of the genes responsible for successive variant types is a consequence of the accumulation of countless mutations. Our continuing structural studies are focusing on a search for regions of constant (or less variable) sequence which we hope will provide clues to the evolution of these antigens and also clarify the features necessary for their integration into the trypanosome surface membrane.

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Enzymatic synthesis of deoxy-5-methylcytidylic acid replacing deoxycytidylic acid in *Xanthomonas oryzae* phage Xp12 DNA

PHAGE Xp12 on *Xanthomonas oryzae* was isolated from the irrigation water of a rice field. In the DNA of this phage, cytosine is completely replaced by 5-methylcytosine¹. Isotope tracing studies on the biosynthesis of this unusual pyrimidine demonstrated that methylation of this cytosine is different from that of the 5-methylcytosine which is found in trace amounts in most plant and animal DNAs. The latter is methylated by methionine. On the other hand, the methyl group of 5-methylcytosine residues of Xp12 is derived from the 3-carbon of serine and not from the thiomethyl carbon of methionine². In this investigation, the synthesis of deoxy-5-methylcytidylic acid (d5MCMP) was found to take place at the level of nucleotide, with deoxycytidylic acid (dCMP) and formaldehyde as substrates in the presence of 5,6,7,8-tetrahydrofolic acid (THFA) and an enzyme preparation from *X. oryzae* infected with Xp12 phage. This reaction seems to account for the synthesis of the unique pyrimidine, 5-methylcytosine, which occurs in the DNA of phage Xp12.

Exponential phase *X. oryzae* strain 604 grown in PS medium³ (potato, 200 g; peptone, 5 g; sucrose, 15 g, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.5 g; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 2 g in 1 l of distilled water) was infected with a fivefold excess of Xp12, which has a latent period of 120 min in these conditions; aeration was continued for 40 min after infection. The cultures were rapidly chilled to 2 °C and the organisms were collected by centrifugation. After washing and resuspension in 0.05 M Tris-HCl buffer, pH 7.5, the cells were disrupted by using a Blackstone Model BP-2 Ultrasonic Probe. Unbroken cells and other particles were removed by centrifugation at 8,000g for 5 min. The supernatants were dialysed against 0.05 M Tris-HCl, pH 7.5 at 4 °C for 4 h. To show the induction by the phage of synthesis of the enzyme with methylating activity, protein synthesis was inhibited by the addition of chloramphenicol (30 $\mu\text{g ml}^{-1}$) just before phage infection. Extracts from uninfected and chloramphenicol-treated bacteria were prepared by the same method from parallel cultures. The protein concentration was approximately 13 mg ml^{-1} as measured by the method of Lowry *et al.*⁴.

The reaction mixture (150 μl) contained 50 μl enzyme extract, 10 μmol dCMP, 0.8 μmol THFA, 2 μmol ^{14}C -formaldehyde (2 m Ci mmol^{-1}), 50 μmol $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 32 μmol 2-mercaptoethanol and 40 μmol Tris-HCl, pH 7.5. The mixtures were incubated at 30 °C for 30 min. The

reaction was stopped by adding 150 μl of 1.0 M sodium acetate, which dissociated 'active formaldehyde' to formaldehyde and tetrahydrofolate⁵, followed by 100 μl of 0.4 M dimedone (in 50% ethanol) to precipitate formaldehyde⁶. The mixture was heated in boiling water for 5 min and the precipitated protein and formaldehyde were removed by centrifugation at 8,000g for 5 min. The supernatant and authentic d5MCMP, prepared from Xp12, were applied respectively to Whatman No. 1 chromatography paper and developed with isopropanol-HCl-H₂O (65:16.6:18.4).

Table 1 shows that the extract of infected cells promotes the synthesis of d5MCMP. A sharp peak of radioactivity was detected at the position corresponding to authentic d5MCMP. For general enzyme assays, the area round the peak was cut out from the paper, placed in a vial, and the radioactivity counted with a Packard Model 3375 scintillation spectrometer. To identify the reaction product, the spot containing the radioactivity was cut out and eluted with water. The eluate was dried and hydrolysed with 50 μl of 90% formic acid at 175 °C for 30 min. Acid hydrolysis of the nucleotide yielded a base with R_f identical to that of authentic 5-methylcytosine in three solvent systems: isopropanol-HCl-H₂O (170:40:39), *n*-butanol-H₂O (86:14) and *n*-butanol-H₂O (86:14) with 5% NH_4OH in air. For further identification, a small amount of the substance obtained by evaporation of the eluate from a chromatogram spot was deaminated with HNO_2 . Deamination was carried out by adding 50 μl of 2 M NaNO_2 and 10 μl of glacial acetic acid to the eluate. After standing overnight at 25 °C, the solution was applied directly to Whatman No. 1 paper. A sample of authentic 5-methylcytosine was treated in the same way. Both the unknown and authentic 5-methylcytosine were found to convert into a substance identical to thymine in its chromatographic properties.

On the basis of these observations, we conclude that an enzyme found in the infected bacteria catalyses the direct methylation of dCMP, but that this enzyme, deoxycytidylate methyltransferase, is absent from uninfected cells. The reaction required the presence of THFA and Mg^{2+} , in addition to dCMP and formaldehyde as substrates. Deoxy-5-methylcytidylic acid can thus be synthesised in phage Xp12-infected *X. oryzae* by the following reaction



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Table 1 Incorporation of H^{14}CHO into deoxy-5-methylcytidylic acid by extracts of Xp12 infected *X. oryzae*

Reaction mixture	Specific activity (nmol d5MCMP formed per mg protein in 30 min)
Complete system*	20.0
Complete system— Mg^{2+}	15.7
Complete system—THFA	1.5
Complete system—dCMP	0.6
Reagent mixture + boiled enzyme†	0.8
Reagent mixture + extract of chloramphenicol-treated cells‡	0.8
Reagent mixture + extract of uninfected cells	1.6

*Complete system as described in text.

†Extract from infected bacteria was heated for 5 min in boiling water.

‡Extract from infected bacteria treated with chloramphenicol. Chloramphenicol was added just before phage infection.

Definitions of free energy levels in biochemical reactions

IN considering the energetics of an enzyme-catalysed reaction it is of interest to enquire into what constitutes the "drive", in a thermodynamic sense, for the reaction, particularly when the enzyme is involved in an energy-coupled process. This often

arises when speculations are made on the operation of a system on the basis of equilibrium constants for the reaction scheme determined on the system, or fragments of it, *in vitro*. In the case of active transport or muscular contraction the free energy for transport or contraction is derived from the hydrolysis of ATP and the overall drive arises because the chemical potential of ATP is greater than that of its products (ADP and P_i) at physiological concentrations. But to understand how the drive is expressed in the mechanism of such processes it is necessary to consider and define the relative free energy levels of the intermediates of the reaction. The free energy and standard free energy as normally defined are not sufficient for this purpose. They both, in general, refer to the system as a whole and are used in the context of free energy changes involving substrates, products and ligands. The drive of the system, in the sense used here, is to be sought in the relative disposition of the free energy levels of an individual (time-averaged) macromolecule and in the manner in which these levels favour (stochastically) a particular direction of the flux in what may be a complicated reaction scheme. We refer to these free energy levels of individual macromolecules as the "basic" free energy levels. To avoid confusion we have termed the overall free energy change of the whole system for a given transition as the "gross" free energy change. The definitions of and relationships between the standard, basic, and gross free energy levels for a system of macromolecules (either in free solution or immobilised) are given below, together with a simple example relevant to muscular contraction. A more mathematically detailed version of this work with more complicated examples for both muscle and active transport has been published elsewhere^{1,2}.

We consider a macromolecule (for example, enzyme) which can exist in a number of states (for example, enzyme-substrate, enzyme-product). In free solution, dilute with respect to enzyme, the chemical potential of the i th state can be written (per molecule and neglecting the activity coefficient) as

$$\mu_i = \mu_i^0 + kT \ln c_i \quad (1)$$

In the case of active transport and of muscle, the macromolecule is bound and the chemical potential can be derived from statistical mechanics¹ as

$$\mu_i = A_i + kT \ln p_i \quad (2)$$

where A_i is the Helmholtz free energy of one macromolecule in the i th state and p_i is the probability of occupancy of this state ($\sum_i p_i = 1$). As this expression is the more general we shall use it throughout, noting for the free solution case that $c_i = bp_i$ and $A_i = \mu_i^0 + kT \ln b$, where b is a constant. (In the bound macromolecule case, A_i is essentially the same as the Gibbs free energy G_i since the two quantities differ only by a negligible pV_i term.)

The free energy change for a transition between two states i and j of a macromolecule E can now be defined (for a kinetic scheme which may have a large number of steps and hence states of E). For generality, consider a transition involving the binding of a ligand (or substrate, and so on)



where E and EL are the i th and the j th states respectively, α_{ij}^* is

the (second order) forward rate constant and α_{ji} is the (first order) reverse rate constant. (The asterisk replaces the prime used previously¹⁻⁴ to denote second order quantities, to prevent confusion with the different assignment of the latter as below.)

The standard free energy change is

$$\Delta\mu_{ij}^0 = A_i + \mu_L^0 - A_j \quad (4)$$

Note that we use Δ in the unconventional sense (initial)—(final), to make most such quantities used below positive.

The basic free energy change is

$$\Delta A'_{ij} = A_i + \mu_L - A_j \quad (5)$$

The gross free energy change is

$$\begin{aligned} \Delta\mu'_{ij} &= \mu_i + \mu_L - \mu_j \\ &= \Delta A'_{ij} + kT \ln(p_i/p_j) \end{aligned} \quad (6)$$

The meaning given to the prime here is as follows: A_i and A_j can be considered as the free energy levels of individual macromolecules, but where binding of a ligand occurs one of them must be "corrected" for the actual (not "standard") ligand concentration if they are to be compared^{3,4}. Thus we can write $A'_E = A_E + \mu_L$ or more generally $A'_i = A_i + \mu_L$, and the use of the prime implies that the A s have been "corrected" where appropriate.

The standard free energy change can be determined if the conventional equilibrium constant ($K^*_{ij} = \alpha^*_{ij}/\alpha_{ji}$) for the transition is known, since

$$\Delta\mu_{ij}^0 = kT \ln K^*_{ij} \quad (7)$$

Note that the asterisks refer to the "second-order" quantities.

It is worth noting that for most biological reactions in free solution the standard and gross free energy changes are, of course simply related to the corresponding standard and "actual" molar Gibbs free energy changes, that is, $-\Delta G_{ij}^0 = N_0 \Delta\mu_{ij}^0$ and $-\Delta G_{ij} = N_0 \Delta\mu'_{ij}$, where $N_0 \equiv$ Avogadro's number and the Δ on the left in both cases is the customary $\Delta \equiv$ final—initial. We approach these changes, however, from the unconventional point of view of single transitions between free energy levels of macromolecular states on a pseudo-isomeric basis and it is appropriate to consider the changes on a "per molecule" basis.

It is easily shown that the basic free energy change is given by

$$\Delta A'_{ij} = kT \ln c_L K^*_{ij} = kT \ln K_{ij} = kT \ln(\alpha_{ij}/\alpha_{ji}) \quad (8)$$

where $K_{ij} = c_L K^*_{ij}$, and $\alpha_{ij}(=c_L \alpha^*_{ij})$ is the pseudo-first-order rate constant for the transition in question.

Thus, "correcting" for ligand concentration yields first-order rate constants and the corresponding (basic) equilibrium constants, which can be treated as if the transition occurred between isomers of the macromolecule.

Since the basic free energy change refers to the difference between the free energies of states of a single macromolecule,

and hence influences its stochastic behaviour, it is of fundamental importance in determining the drive in a reaction sequence. A diagram of the relative basic free energy levels (see Fig. 1) is, as shown above, closely related to the kinetic description in terms of first-order rate constants (though energy barriers between states are not shown in Fig. 1). Thus a downwards change favours flux through the system in the direction of the

basic change by a term $(kT \ln p_i/p_j)$ in equation (6). This is a typical "concentration" term of purely entropic origin. A situation may thus arise where the basic free energy change between two successive states is small, but the gross free energy change is large. (This can occur in an enzymatic cycle when the transition $i \rightarrow j$ is the rate-limiting step, so that in the steady state $p_i \gg p_j$. An example of this is given below.)

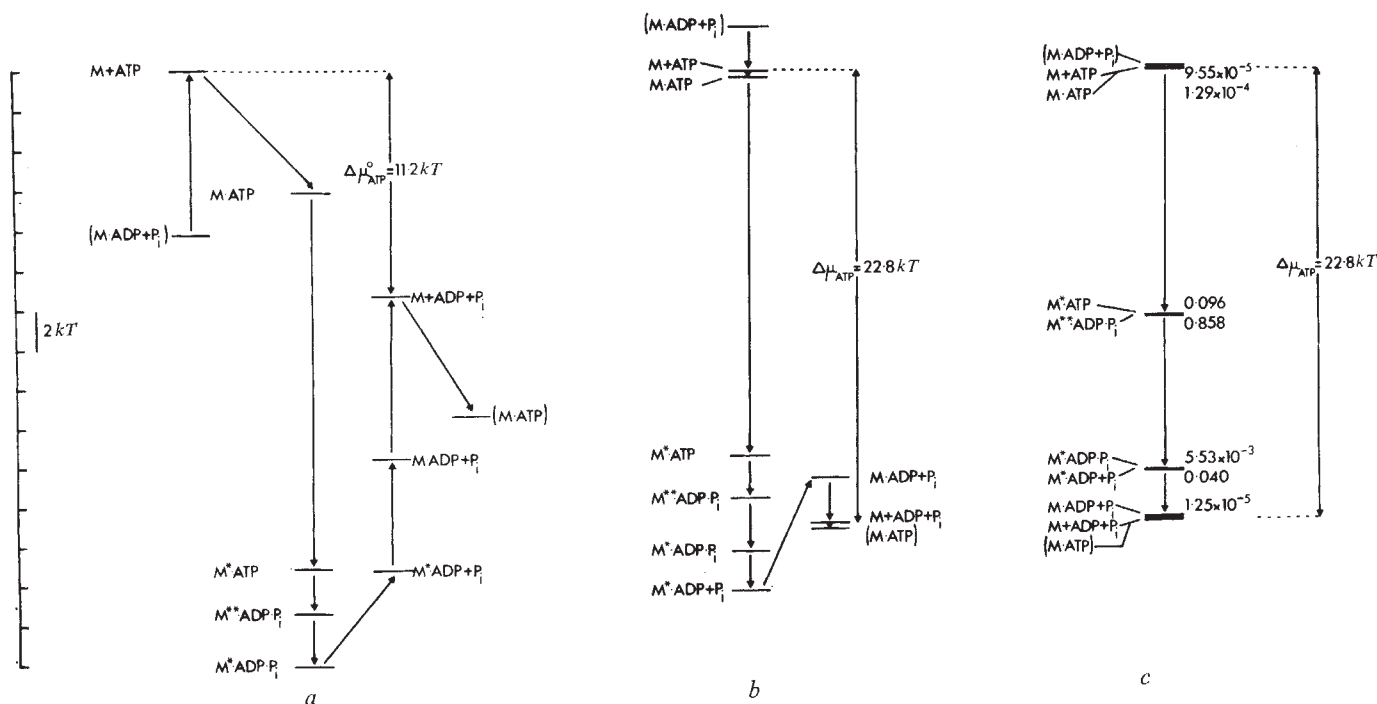


Fig. 1 Free energy levels for the intermediates in the hydrolysis of ATP by myosin *SI* (*M*) at 20 °C. Concentrations of ATP, ADP and P_i were chosen as 3 mM, 0.03 mM and 1 mM respectively. $\Delta\mu^\circ_{\text{ATP}}$ was taken as 11.2 kT. $M + \text{ATP}$ was arbitrarily set at the same free energy level in (a), (b) and (c). Intermediates in parentheses belong to sets of levels immediately above or below those shown in the figure. The numbers opposite gross free energy levels (c) are the steady-state occupancies (p_i).

overall drive ($\text{ATP} \rightarrow \text{ADP} + P_i$) whereas an upwards change opposes it. It is the downward basic changes that constitute the stochastic drive at the level of the intermediates in a reaction.

As for the standard free energy, in the case of transitions where the binding of ligands, and so on is not involved (isomeric transitions such as $ES \rightleftharpoons EP$), the standard free energy change is easily seen to be numerically identical to the basic change. But in non-isomeric transitions the two are by no means the same; in fact, the standard free energy change is of little interest as it refers to ligand, and so on at molar (that is "standard") concentration. Similarly the basic (isomeric) equilibrium constant K_{ij} is more relevant to actual conditions than the "standard" equilibrium constant K°_{ij} .

The gross free energy change can be calculated when the probabilities (or concentrations) of the intermediates are known, in addition to the equilibrium constants. It gives the Gibbs free energy change that actually occurs in a transition when the whole system is considered. This is to be contrasted with the basic change description in which a single macromolecule undergoes transitions stochastically⁵ and is "unaware" of the rest of the system. Thus the gross change is related to the overall flux and entropy production in a transition^{1,2}. It should be noted that, for given substrate, and so on, concentrations, the basic changes and levels are invariant whereas the gross changes depend on the exact conditions of the system (in the steady state or during transients).

The gross free energy change for a transition differs from the

Suppose now that these states are coupled to another reaction, to transport or, in the case of muscle, to the performance of mechanical work; it might be deduced that the coupled process is being "driven by the concentration term". This would, in general, be incorrect; the efficiency and flux of a coupled process that is far from equilibrium result from the detailed kinetics of the entire cycle of the driving reaction and the kinetic constraints imposed on it by the coupled process.

For an enzyme which hydrolyses ATP, in each completed cycle the decrease in free energy is given by the sum of either the basic or the gross changes, that is

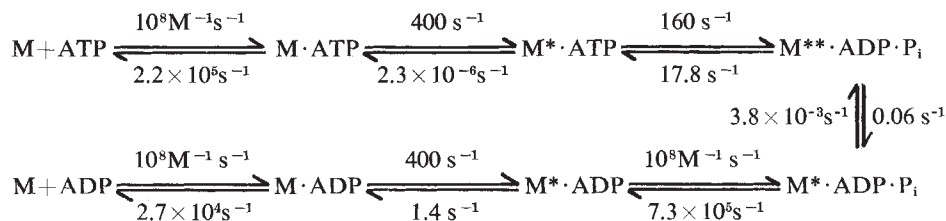
$$\Delta\mu_{\text{ATP}} = \sum \Delta A'_{ij} = \sum \Delta\mu'_{ij} > 0 \quad (9)$$

The free energy decrease per molecule of ATP hydrolysed is given by

$$\Delta\mu_{\text{ATP}} = \Delta\mu^\circ_{\text{ATP}} + kT \ln(c_{\text{ATP}}/c_{\text{ADP}}c_{P_i}) \quad (10)$$

Here again the standard free energy change is of no direct interest.

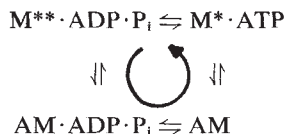
A comparatively simple example related to muscular contraction will now be given to illustrate the different definitions. (More complicated models both of active transport and of muscle are discussed in refs 1-3). The kinetic scheme for the hydrolysis of ATP by myosin subfragment 1 (*M*) has been determined⁶⁻⁸ as



where the rate constants for binding ATP, ADP and P_i have been chosen as $10^8 \text{ M}^{-1} \text{ s}^{-1}$ and the forward rate constant for the third step represents the minimum likely value. Assuming reasonable values for the physiological concentrations of ATP, ADP and P_i , the relative free energy levels between states can be calculated, taking the steady-state case for the gross free energy changes (Fig. 1).

As noted above, the magnitude of free energy changes for the standard and basic diagrams coincide only for isomeric transitions (for example, $\text{M} \cdot \text{ATP} \rightleftharpoons \text{M}^* \cdot \text{ATP}$). As regards the difference between the basic and gross diagrams, the former has both upward and downward transitions, the principal downward changes being for the two-step binding of ATP. The free energy changes for the gross diagram are all downward. This is a natural consequence of the steady state in a single cycle in which the flux through the system is unidirectional¹. The largest free energy change is for the two-step binding of ATP in both the basic and gross diagrams, but in the gross diagram there is not inconsiderable change at the rate-limiting step ($\text{M}^{**} \cdot \text{ADP} \cdot \text{P}_i \rightleftharpoons \text{M}^* \cdot \text{ADP} \cdot \text{P}_i$). This is a consequence of the build-up of the occupancy of the state preceding the rate-limiting step. In the gross diagram several pairs of states are at nearly the same free energy level indicating that, in the steady-state conditions used, these pairs are essentially in equilibrium (indeed, when this is considered in conjunction with the sparsity of occupation of the states M , $\text{M} \cdot \text{ATP}$ and $\text{M} \cdot \text{ADP}$, the scheme reduces essentially to a two-state energetic and kinetic process consisting of $\text{M}^* \cdot \text{ATP}$ "lumped" with $\text{M}^{**} \cdot \text{ADP} \cdot \text{P}_i$ and $\text{M}^* \cdot \text{ADP} \cdot \text{P}_i$ "lumped" with $\text{M} \cdot \text{ADP}$). In all the diagrams, for each cycle completed, the subsystem or system progresses to another, identical, set of levels shifted downwards from the previous set by $\Delta\mu_{\text{ATP}}$ ($\Delta\mu^0_{\text{ATP}}$ for the standard diagram).

The SI scheme provides considerable scope for speculation about the basic free energy levels of the cyclic interaction of myosin with actin in the crossbridge mechanism of muscular contraction. Unfortunately full details of the corresponding scheme in the presence of actin are not yet known; also there are serious difficulties in translating a kinetic scheme derived for free solution to that for the intact myofibrillar matrix¹⁰. Nevertheless, modifying slightly a much simplified scheme for actomyosin¹¹,



the M and AM states correspond respectively to detached and attached states of crossbridges in muscle and the drive (and directionality) of contraction must be reflected in the downward progression of basic free energy levels between successive AM states in the (biochemical) actomyosin scheme^{3,12}. Large downward changes elsewhere in the cycle could lead to a waste of free energy in the gross free energy representation for intact muscle. It seems that such changes may not exist, at least in the basic representation for the actomyosin scheme (H. D. White and E. W. Taylor, in preparation); for example, the dissociation constant and hence the basic free energy change for $\text{AM} + \text{ATP} \rightleftharpoons \text{A} + \text{M}^* \cdot \text{ATP}$ is small, the large binding energy for ATP to myosin being offset by that of actin to

myosin¹³. Furthermore, the basic change for $\text{M}^* \cdot \text{ATP} \rightleftharpoons \text{M}^* \cdot \text{ADP} \cdot \text{P}_i$ is comparatively small (Fig. 1). It is therefore possible to regard $\text{M}^{**} \cdot \text{ADP} \cdot \text{P}_i$ as a "high energy state", in relation to the last attached state (AM) in the scheme above, in the sense that the (probably) large downward basic free energy change between them serves as the drive for contraction (although it seems unlikely that all the available energy from ATP hydrolysis is conserved in the state $\text{M}^{**} \cdot \text{ADP} \cdot \text{P}_i$; compare ref. 14).

Gray¹⁵ has argued on quantum mechanical grounds for an irreversible step in the crossbridge cycle. This invites the comment that it all depends on what you mean by irreversible; as we have argued above, it does seem that a necessary requirement for a high efficiency in muscle is a large downward basic free energy change from the state prior to attachment to the last attached state (measured in the latter in the absence of mechanical constraints), but that does not necessarily mean that the transitions would be entirely irreversible. However, a full treatment of this kind of problem involves the averaging of rate constants and free energy levels over the distribution of crossbridges in each attached state³. The actual system considered (for comparison with experiment) is not a single crossbridge but a macroscopic ensemble of crossbridges under a potentially wide distribution of mechanical constraints; there is no simple way of introducing these constraints, for example, by introducing a work-dependent opposing thermodynamic flux into the scheme (compare ref. 16). We hope to return to this problem in a future paper.

Finally, it should be stressed that a basic free energy diagram gives no more than a useful summary of the energetic situation for a single macromolecule. This may be useful in model-building⁹ or in comparing different systems, but a full description of the energetics of a system in particular conditions requires a detailed knowledge of the kinetics of the system to generate (and understand) the gross free energy diagram.

This subject is treated in more detail and in a much more general context elsewhere⁹.

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reviews

Introducing HLA

Heather M. Dick

The HLA System: An Introductory Survey. (Monographs in Human Genetics, Vol. 7.) By A. Svegaard *et al.* Pp. viii+103. (Karger: Basel, London and New York, 1975.) SF/DM 38; \$14.75.

THIS is an excellent little volume which sets out with a refreshing lack of pretentiousness to give a simple introduction to the HLA system. The authors certainly achieve their main purpose and offer a text which will be invaluable to those who wish to gain access to exciting new developments.

The HLA antigens are no longer the specialised subject of a few devoted transplantation workers. The wide range of problems in biology and clinical medicine which can be related to the major histocompatibility system is evidence of the explosion of knowledge which has occurred in this subject.

After a clear exposition of the components of the HLA system, in particular the genetics, we are led straight into the most recent and exciting work on the similarities between human and mouse immune response mechanisms. The section on biochemistry is brief—reflecting the paucity of information on this aspect until very recently, but introducing the reader immediately to newer and more rewarding developments on the structure of HLA substance. The practical applications of studies on HLA antigen frequencies are dealt with adequately enough for the general reader.

The authors are perhaps too modest in not including more of their own excellent work on the statistical and technical problems of estimating associations between specific HLA antigens and diseases. They also shy away from a detailed discussion of the mechanisms which might be responsible for such associations. Although they list several impeccable reference sources dealing with this problem, it would probably be helpful to readers who are not intimately involved in the current work to have the arguments for and against the suggested hypotheses presented in some detail. Perhaps the authors felt they had already made it obvious that their preference was for the genetically de-

termined "immune-response gene" hypothesis, but it would not weaken their argument to expose the other hypotheses of molecular mimicry, or HLA as virus receptor to the cold light of print.

The final section on methodology is adequate to provide background information for those not directly concerned with technique, although the difficulties of explaining MLC typing to an uninitiated audience are painfully obvious. There is a very full bibliography to complete the text.

The material presented is up-to-date and the authors are to be congratulated

on presenting a useful and readable volume which includes so much contemporary material. Textual misprints are a minor source of annoyance. The occasional lapses from colloquial English by the team of Danish authors might have been edited out, but do not detract in any way from the value of the book. Certainly, this reviewer could not hope to do as well in Danish. □

Heather Dick is a Consultant in Clinical Immunology at Glasgow Royal Infirmary, in charge of histocompatibility services for Glasgow and the west of Scotland.

Hückel model

The HMO Model and Its Application. Vol. 1: Basis and Manipulation. By Edgar Heilbronner and Hans Bock. Pp. xv+454. (Wiley: London and New York; Verlag Chemie: Weinheim, February 1976.) £16; \$35.20.

THIS is the first volume of a three-volume treatise which is intended to acquaint "the practically oriented chemist having a relatively modest mathematical background" with the principles of the Hückel molecular orbital (HMO) model and with the simple techniques with which it can be used to obtain approximate but very useful qualitative results without recourse to a computer. Volume 1 contains all the theory and applications, and 250 problems; volume 2 will contain the solutions to the problems; and volume 3 will contain HMOs and derived quantities for simple π -electron systems.

This all seems splendid, but I cannot recommend the practically oriented chemist to rush out for his copy, and I doubt whether he would if I did. One reason is that this volume alone costs £16, so that the whole set is going to add up to a lot of money. Furthermore, for all that the authors claim to "fit the intuitive conceptual world of the chemist", there is not really very much discussion of the chemistry. Coulomb integrals, for instance, appear as numbers, with due acknowledgement to Streitwieser but no attempt to relate

their values to the chemist's notion of electronegativity, which indeed is not mentioned anywhere. More seriously, the treatment of reactions is now badly out of date, the book being an unrevised translation of the 1968 German edition; it is a great pity that the opportunity was not taken to add, for example, an account of the frontier orbital method, which has proved so fruitful in recent years.

Other criticisms, which I make with some reluctance because I am in sympathy with the authors' intentions and find a great deal of useful material here, are that the style is intensely turgid and polysyllabic, and that some mathematical derivations are given in painful detail, whereas other results are quoted without the proof which might quite easily have been given. Finally, I disagree myself with the authors' policy of saying very little indeed about the deficiencies of the HMO model and about the way they can be overcome in more elaborate methods.

I do not underestimate the difficulty of putting molecular orbital theory over to the non-mathematical chemist. This is not a bad book by any means, but it is too heavy, too expensive and too late.

A. J. Stone

Dr Stone is an Assistant Director of Research at the Chemical Laboratories, University of Cambridge, UK.

Cell handbook

Cell Biology. (Biological Handbooks, 1.) Compiled and Edited by Philip L. Altman and Dorothy Dittmer. Pp. xix+454. (Federation of American Societies for Experimental Biology: Bethesda, Maryland, 1976.)

FROM time to time a new book appears which is not simply a rehash of those that have gone before. Until now, biology has lacked its "Beilstein" and it is often a time-consuming business to find some small piece of information which you know has been published. This book compresses the maximum amount of factual information into the minimal space and at the same time gives key references from which a quick general overview of the field of interest may be made. To give some examples, do you want to know what nutritional mutants of mammalian cells are available; 31 types are listed and 40 references are given to the field, all in the space of 2 pages. Are you interested in cloning mammalian cells; 52 examples are given and 49 references in only 3 pages. How long would it take to find the oxygen consumption of 618 different mammalian tissues; it is all here

with 198 references compressed into 17 pages. As this one volume extends to 400 pages, excluding appendices and a full index, the amount of information available is remarkable.

Some sample headings, similar in range to those listed above, will give an indication of the scope of this miniature encyclopaedia: resting cell membrane potentials, rheological properties of cells, changes in cell surface membranes with malignant transformation, permeability of inner mitochondrial membrane to anionic substrates, gross chemical composition of microsomes, occurrence of microfilaments in non-muscle cells and tissues, DNA content of cell nuclei. Obviously this is not a book that can be read but it is a book which most biologists are likely to treasure. Inevitably the choice of key references is open to criticism and at times the choice seems somewhat parochial. Nevertheless sufficient are given to enable the research worker to get off to a flying start.

This series is likely to be a must in many libraries.

T. S. Work

Dr Work is Head of the Division of Biochemistry at the National Institute for Medical Research, London, UK.

Somatic cell genetics

Variation, Senescence and Neoplasia in Cultured Somatic Cells. (A Commonwealth Fund Book.) By John W. Littlefield. Pp. xi+163. (Harvard University: Cambridge, Massachusetts and London, 1976.) £6.15.

THOSE of us who work in the field that is now somewhat pretentiously called somatic cell genetics owe John Littlefield a great deal. This little book puts us further in his debt. It is based on a course taught by him at Harvard over a number of years. The students who were exposed to this teaching can count themselves fortunate. The information presented is constantly subjected to the sort of critical assessment that can be provided only by someone who has himself worked long and intelligently in the field.

As its title indicates, the book has three main themes. The chapters dealing with genetic and other forms of variation in somatic cells and those dealing with the phenomenon of 'senescence' *in vitro* are difficult to flaw. The discussion of senescence, in particular, is informed by a degree of perception that I have not found

matched in any other published account of this subject.

I hope I may be forgiven for saying that I found the treatment of neoplasia less good. I think this may be because the author has not himself had so much experience of work with tumours; whereas, of course, he has made monumental contributions to the study of cells *in vitro*. In discussing experiments on somatic cells growing *in vitro*, he is very conscious of the quantitative aspects of the work he is describing, and especially of quantitative deficiencies in the evidence presented. His assessment of experiments dealing with the formation of tumours is, however, less sure. If he had treated the relationship between 'transformation' and tumorigenicity with the same rigour as characterises his analysis of mutagenesis in somatic cells, I doubt whether he would have written: (Established) "heteroploid lines appear to have lost growth control and to have become neoplastic". Even so, there's no chapter in this book that isn't worth reading, and there is, as a very important bonus, an absolutely splendid bibliography.

Henry Harris

Henry Harris is Professor of Pathology at the University of Oxford, UK.

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obituary

The recent premature and unexpected death of **James Olds** deprives physiological psychology of one of its most distinguished workers and the neurosciences of a man whose experimental work cannot be ignored in any serious behavioural theory. His major discovery provoked controversy from the time of its first report and although he made substantial contributions himself, many related issues are unresolved at the time of his death.

In 1953 Olds and P. M. Milner, working in D. O. Hebb's laboratory at McGill reported that rats with electrodes in lateral hypothalamic and septal sites would press a lever to deliver trains of stimuli to their own brains. Electrical self-stimulation revealed the existence of powerful central reward mechanisms and immediately raised difficulties for theories in which behaviour is initiated only to mitigate the aversive consequences of internal disequilibrium. The phenomenon had not been predicted by theories current at the time (but the aversive effects of brain stimulation had been looked for and found the previous year by Delgado, Roberts and Miller) and although he had considered the existence of central reward processes, Olds was working on the 'arousing' effects of electrical stimulation when he made his discovery. Noting a tendency for one rat to return to a point where he had previously been stimulated, Olds and Milner were quick

to exploit this phenomenon with an operant conditioning apparatus. Many researchers must previously have seen sniffing and approach behaviours following stimulation through implanted electrodes. The originality of the discovery lay in seeing the possible significance of this behaviour and examining it in a Skinner box.

In succeeding years Olds made major contributions to understanding self-stimulation behaviour. With Travis and Schwing, and later with M. E. Olds, he prepared what are still the most extensive maps of approach and avoidance areas in the rat brain. With Travis, Yuwiler and others he initiated investigations of the possible neurochemical basis of the phenomenon. His suggestion in 1960 that the major tranquillizers act specifically on the mechanisms of approach behaviour has recently attracted new interest in the light of the action of these drugs on dopaminergic mechanisms. Increasingly Olds became interested in single cell recordings as a method of investigation, but he never felt that these had given the answer he really wanted, the anatomical identity of the reward mechanism itself.

Olds' writings on self-stimulation included many provocative theories. He took an intense interest in new ideas and findings which might be relevant to the problem, and in recent years had been preoccupied with the possible role of monoamine neurones in reinforce-

ment. To the end of his life he remained at the centre of controversies concerning the functional significance of this behaviour and its neuroanatomical basis, and in retrospect it was perhaps fortunate that so much work in this field was gathered together at the Beerse conference in 1975 (*Brain Stimulation Reward*, edited by A. Wauquier and E. T. Rolls, Elsevier). James Olds must have impressed many of those present as the man best able to draw together the various strands of evidence in a coherent synthesis. Particular interest attaches therefore not only to his contributions to this symposium but also to the as yet unpublished monograph on Drives and Reinforcements completed a year before his death.

Olds modestly maintained that if he had not discovered electrical self-stimulation it would soon have been uncovered by someone else. This may be doubted, but even if true, few workers could have contributed his enthusiasm and imagination to developing a whole new field of psychophysiological work. He was a man who was willing to consider unusual concepts and paradoxical ideas but pursued them with great clarity. Everyone in the field will feel the loss of his critical intelligence and many will also acknowledge a debt of gratitude for his personal encouragement of their own efforts.

T. J. Crow

announcements

Appointments

Dr H. S. Bedson to the Chair of Medical Virology at the University of Birmingham.

Dr Leo A. Kaprio has been reappointed as Regional Director for Europe of the WHO.

Mr E. S. Booth, chairman of the Yorkshire Electricity Board, as the next President of the IEE.

Professor A. Lazenby, at present Vice-Chancellor of the University of New England, NSW, as the next Director of the Grassland Institute.

Professor D. R. Stranks, of the University of Melbourne, as Vice-Chancellor of the University of Adelaide.

Professor E. W. J. Mitchell, Head of the Department of Physics, as Deputy Vice Chancellor of the University of Reading.

Dr Derek Ogston, Reader in Medicine as Regius Professor of Physiology at the University of Aberdeen.

Dr Roger Grice, of Cambridge University, as Professor of Physical Chemistry at Manchester University.

Professor John Fraser Scott, of the University of Sussex, has been appointed Vice-Chancellor of La Trobe University, Melbourne.

Awards

The Faraday Division of the Chemical Society has awarded the 1976 Marlowe

Medal to **Dr James J. Burton** of the Exxon Research and Engineering Company for his theoretical work on microclusters.

The Finsen awards for 1976 have been made to **S. B. Hendricks** (Control of plant development by light), **H. F. Blum** (Skin and Cancer Hospital, Philadelphia, USA) (Photodynamic action and carcinogenesis by UV light), and **D. Shugar** (University of Warsaw, Poland) (Photochemistry and the structure of nucleic acids and proteins).

The SRC has awarded Senior Fellowships to **Dr A. Boksenberg**, of the Department of Physics, University College, London; **Professor A. Car-**

ington of the Department of Chemistry, University of Southampton; **Professor B. C. Clarke** of the Department of Genetics, University of Nottingham; **Dr P. H. Gaskell** of Pilkington Bros Ltd and the University of Cambridge; **Professor C. A. R. Hoare** of Queens University, Belfast; **Professor E. C. Zeeman** of the Department of Mathematics, Warwick University.

Meetings

October 11–15, **Clean Air**, Edinburgh (The Press and Information Officer, National Society for Clean Air, 136 North Street, Brighton BN1 1RG).

November 4–5, **Mutilation of Animals**, London (Symposium Organising Secretary, RSPCA Headquarters, Causeway, Horsham, Sussex RH12 1HG, UK).

November 8–10, **Manufacture of Superconducting Materials**, Port Chester, New York (Conference Coordinator, Technical Divisions and Activities, American Society for Metals, Metals Park, Ohio 44073).

November 17–18, **Agricultural Yield and Efficiency**, London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

December 1–2, **Food Additives in the Balance**, Harrogate (Dr D. Howling, c/o Technical Division, CPC (United Kingdom) Ltd, Trafford Park Road, Trafford Park, Manchester M17 1PA, UK).

January 5–7, **Solid State Physics**, Manchester (Deadline for contributions: November 1) (Professor H. E. Hall, Department of Physics, University of Manchester, Manchester M13 9PL, UK).

January 18–19, **Optical Production Technology**, London (Mrs R. G. Keiller, SIRA Institute Ltd, South Hill, Chislehurst, Kent BR7 5EH, UK).

January 25, **Energy Transfer Mechanisms, Dosimetry, Dose-Response Relationships**, London (Professor J. H. Martin, Department of Medical Biophysics, Blackness Laboratory, University of Dundee, Dundee, UK).

February 16–18, **4th International Congress on Mutations: Biology and Society**, Paris (Congrès Service, 1, rue Jules Lefèvre, F 75009, Paris, France).

March 17–18, **Perinatal Care**, New York (Dr Raymond L. Vande Wiele, Co-chairman of Symposium on Future Directions in Perinatal Care, College

Person to Person

Nominations for the 4th Royal Society Esso Award for the Conservation of Energy should be sent to the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG by February 7, 1977. The award consists of a gold medal and a prize of £1,000. It is for outstanding contributions which in the opinion of the Council of the Royal Society will lead to more efficient conversion or use of energy.

Dr R. P. Sheldon, University of Bradford, is preparing a report on the research which is going on in polymers/plastics in medicine (including surgery, dentistry, etc.) in the UK. Anyone who is working in this area is asked to contact Dr Sheldon giving brief details.

Applications are invited for Johanoff Fellowships (worth \$15,000), to spend a year at the Mario Negri Institute in Milan doing cancer chemotherapy and/or immunology, cardiovascular pharmacology, neuropsychopharmacology or drug metabolism. Candidates should be non-Italian scientists of established reputation. Apply to The Johanoff Fellowship Committee, Istituto di Recherche Farmacologiche Mario Negri, Via Eritrea 62, 20157 Milan by January 31.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

of Physicians and Surgeons of Columbia University, Department of Obstetrics and Gynecology, 630 West 168th St, New York, N.Y. 10032).

March 29–31, **Neuromuscular Disorders**, Bath (Dr G. G. Lune, Department of Biochemistry, University of Bath, Bath BA2 7AY, UK).

April 17–30, **Chemically Induced Magnetic Polarisation**, Sogesta, Urbino, Italy (Professor L. T. Muus, Chemistry Institute, 140 Langelandsgade, DK-8000 Aarhus C, Denmark).

April 23–28, **Annual Meeting of the American Ceramic Society**, Chicago (Deadline for abstracts: November 20) Dr D. N. Winslow, School of Civil Engineering, Purdue University, West Lafayette, Indiana 47907).

April 25–29, **11th International Sym-**

posium on the Remote Sensing of the Environment, Ann Arbor, Michigan (Dr Jerald J. Cook, Environmental Research Institute of Michigan, PO Box 618, Ann Arbor, Michigan 48107).

May 1–7, **Stereochemistry**, Burgstock, Switzerland (Professor P. Pino, Laboratorium für Technische Chemie, ETH, Universitätsstr. 16, CH-8092, Zurich, Switzerland).

May 23–27, **Safety at Sea**, London (The Conference Secretary, WEMT 1977, 10 Upper Belgrave Street, London SW1X 8BQ, UK).

August 29–September 1, **Drugs Affecting Lipid Metabolism**, Philadelphia (Deadline for abstracts: April 15) (Dr William L. Holmes, Scientific Secretary, Symposium on Drugs Affecting Lipid Metabolism, Lankenau Hospital, Philadelphia, Pennsylvania 19151).

August 30–September 2, **Biological Activity and Chemical Structure**, Noordwijkerhout, the Netherlands (Secretariat Department, c/o Merck Sharpe and Dohme B.V., Waarderweg 39, PO Box 581, Haarlem, the Netherlands).

August 31–September 2, **Astronomy and Astrophysics**, Christchurch, New Zealand (Astrophysics Conference Secretary, Department of Physics, University of Canterbury, Private Bag, Christchurch, New Zealand).

September 4–9, **13th International Symposium on Free Radicals**, Lyndhurst, Hampshire, UK (Professor A. Carrington, Chemistry Department, The University, Southampton SO9 5NH, UK).

September 11–16, **Pathology**, London (Professor A. Munro Neville, Vith European Congress of Pathology, Institute of Cancer Research, Fulham Road, London SW3 6JB, UK).

September 12–14, **Electron Microscopy and Analysis**, Glasgow (The Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

September 27–29, **Power Semiconductors and their Applications**, London (Deadline for abstracts: November 1) (Press Officer, IEE, Savoy Place, London WC2R 0BL, UK).

October 24–26, 1977, **Erosion: Prevention and Useful Applications**, Vail, Colorado (Deadline for abstracts: March 30) (Dr William F. Adler, Effects Technology, Inc., 5383 Hollister Avenue, Santa Barbara, California 93111).